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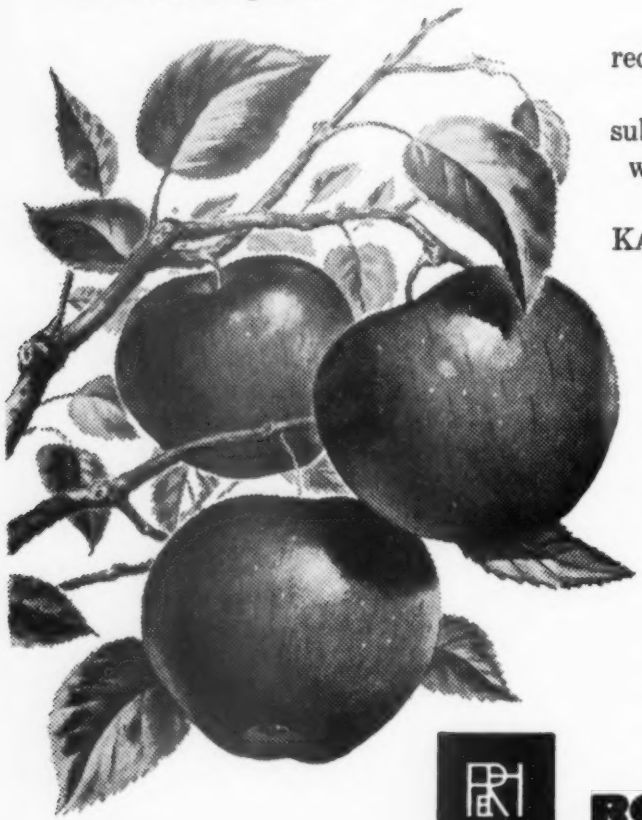

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FIFTIETH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY,

BLOOMINGTON, INDIANA, AUGUST 24, 25, 26, 27, 28, 1958

Uptake of antibiotic GS1 by seeds and plants. ARK, PETER A., AND J. DEKKER. The antibiotic GS1 (Product of Charles Pfizer and Co., Inc.) readily penetrates both pea and squash seeds soaked 24 hours, and can be demonstrated in the cotyledons after washing and removal of the seed coat. Good control was obtainable on internally borne *Fusarium solani* f. *cucurbitae* and *Ascochyta pisi* with, respectively, 50 and 100 ppm of the antibiotic, and no phytotoxicity was apparent. In bean, cucumber, and barley plants the upward movement of the antibiotic could be demonstrated on bioassay plates in the plant juice only when the antibiotic was taken up by the roots from solutions containing 200 ppm. At lower concentrations of the antibiotic, detection was possible only by aqueous distillation of the leaves. When the antibiotic was placed in contact with bean leaf sap for thirty minutes, no inhibitory activity could be detected unless the juice had been adjusted to pH 4.5 before adding the antibiotic.

The serological relationships of potato viruses S and M, and the carnation latent virus. BAGNALL, R. H., C. WETTER, AND R. H. LARSON. Potato viruses S and M have a small antigenic fraction in common, and each has a separate fraction in common with the carnation latent virus. Each of these 3 viruses has a major antigenic fraction distinct from the others. The 2 potato viruses have significant differences in host range, symptomatology, and physical properties, and have no known hosts in common with the carnation latent virus. The 3 viruses probably had a common ancestor, but the differences seem to warrant classification as separate species or subspecies rather than as strains.

A difference in the pathogenicity of the citrus nematode from trifoliate orange and sweet orange roots. BAINES, R. C., O. F. CLARKE, AND J. W. CAMERON. The pathogenicity of citrus nematode larvae obtained from *Poncirus trifoliata* roots in 3 orchards and from sweet orange roots in 1 orchard was tested on small seedlings from self-pollinated flowers of Fawcett-Webber and Pomeroy varieties of *P. trifoliata* and from open-pollinated flowers of Standard sour orange. Each variety-inoculum class, 6 pots of 4–5 seedlings each, received about 20,000 larvae per pot. The 3 lots of nematodes from *P. trifoliata* roots infested 90% of the *P. trifoliata* seedlings slightly, and 10% moderately. Only small differences occurred in the pathogenicity of the nematodes from the 3 sources of *P. trifoliata* and in the susceptibility of the 2 varieties of *P. trifoliata*. Nematodes from sweet orange roots slightly infested 1% of *P. trifoliata* seedlings, leaving 99% uninfested. All sources of inoculum infested all Standard sour orange seedlings moderately or severely.

Synthesis of mycorrhiza with pure cultures of Rhizophagus. BARRETT, JAMES T. Synthesis tests using pure cultures of a widely occurring root endophyte on several plant species gave positive results, with varying infestation and mycorrhizal development. The tests were made mostly with 5 of 10 isolates of the fungus, introduced in pots of sterile sand and sand-soil mixtures. All isolates produced the same type of host-endophyte relationship in the same host plant. Gallaud (1904) described 2 types of the endophyte: the Arum, or intercellular, and the Paris, or intracellular. The tests reported here do not confirm Gallaud's findings. For examples: an isolate that developed the Arum type in *Zea mays* produced the Paris type in *Solanum tuberosum*, and a peleton type in Iris. A Paris type from Heterocallis developed the Arum type in *Zea mays*. These results indicate that the host, not the endophyte, determines the host-fungus relationships. Synthesis tests on *Solanum tuberosum*, grown from both seed and tubers, using 7 isolates of the fungus, gave positive results and revealed this species to be less susceptible than several other plants used. No infestation of stolons or tubers by the endophyte was detected. These synthesis tests furnish evidence that the cultures used are the *Rhizophagus* endophyte.

Production of ferment gum disease in Marsh grapefruit trees by fungus inoculation. CALAVAN, E. C., AND D. W. CHRISTIANSEN. Ferment gum, a major disease of grapefruit (*Citrus paradisi* Macf.) trees in California, is characterized as follows: exudation of clear or frothy, amber to reddish gum from blisters under the bark; zones of firm, buff, malodorous, nectrotic wood, with pink to orange margins; areas of brown wood rot extending from wound infection sites; and general deterioration of infected branches. Except for a difference in the type of wood rot, ferment gum disease resembles Rio Grande gummosis, attributed in Texas to *Diplodia natalensis*. In California, a wood-rotting basidiomycete having whorled clamp connections in young hyphae and branched rhizomorphs in old cultures was shown to be a primary causal organism of ferment gum in grapefruit trees. Typical symptoms of ferment gum developed within 13 months in 37 of 69 wound inoculations with this fungus, which was reisolated from brown rotten wood near the inoculation sites. Of the 11 organisms (bacteria, fungi, and actinomycetes) isolated from ferment gum lesions and inoculated into wounds in Marsh grapefruit trees, only the wood-rotting fungus with whorled clamp connections reproduced the ferment gum syndrome. The causal organism formed oidia in cultures, but fungus fruiting structures and spores have not been found.

Pathogen-free lilies from cultured bulb-scales. CHANDLER, PHILIP A., AND J. G. BALD. Adventitious bulblets were produced on surface-sterilized scales of *Lilium longiflorum* cultured in tubes on potato dextrose agar. The best time to sample bulb scales for treatment was during or soon after flowering. Later, diminishing numbers of bulb scales were made aseptic by washing in running water and surface-sterilizing. Organisms entered the scales at the tip and base. Many of these organisms, including *Fusarium* spp., were killed by hot-water treatment at 125°F for 30 minutes, the scales were unharmed, and a maximum yield of aseptic bulbils was obtained when the hot-water-treated scales were cultured—up to 50% in some lots. The treatment was applied successfully to varieties of *L. longiflorum* other than Croft, and to *L. candidum*. The combination of hot-water treatment and culturing is a possible means of obtaining elite stocks of established lily varieties for commercial production.

Inclusion bodies in citrus. CHILDS, J. F. L. In limited examinations, inclusion bodies typical of those associated with virus infections of herbaceous plants were found in the bark of *Poncirus trifoliata* free of psorosis, tristeza, and exocortis, as indicated by standard index tests. They were abundant in seed cotyledons of Sampson tangelo (*Citrus paradisi* × *C. reticulata*) and also grapefruit (*C. paradisi*), variety unidentified, that exhibited symptoms of stubborn disease; were less abundant in seeds of Sampson tangelo without stubborn symptoms; and were scarce in seeds of 2 seedling sweet orange (*C. sinensis*) trees. In F.A.A.-fixed bark and seeds, inclusions and nucleoli stained

as follows: red with Cartwright, red with acid fuchsin-fast green, and purple-blue with Giemsa. Inclusions in seeds gave a weak arginine reaction with Carver's modified Sakaguchi test, a negative Feulgen test, and negative reactions for tyrosine (Serra's modified Millon test), aldehydes (Schiff, phloroglucinol, and ammoniacal $AgNO_3$), fat (Sudan IV), tannins (ferric chloride), and cellulose (zinc-chloro-iodide). Inclusions in F.A.A.-fixed seeds stained pink with ruthenium-red, orange with I-K-I, and grey-blue with lacmoid. Unfixed inclusions were not stained.

Protective role of the pseudosclerotium of Armillaria mellea against attack by Trichoderma viride. DARLEY, ELLIS F., AND WESLEY D. WILBUR. Previous work suggested that the pseudosclerotium of *A. mellea* resists attack by *T. viride*, and that its protective mechanism is altered after soil fumigation with CS_2. This suggested that mechanical destruction of the pseudosclerotium should permit invasion by *Trichoderma* in the absence of CS_2. To test this, artificially infected sections of citrus root and uninfected roots were buried for periods up to 3 years in separate sites in a naturally infested orchard. The bark (and pseudosclerotium on infected roots) had been left undisturbed on 1 group of each class of roots; stripped from a second group; and stripped from a third group, and the roots split. *Armillaria* was viable in all but 1 of roots buried 1, 17, and 36 months, regardless of the damage inflicted on the pseudosclerotium. The pseudosclerotial layer had reformed incompletely on roots buried 17 and 36 months. Bacteria, various fungi, including *Trichoderma*, and even *Armillaria* were isolated from the previously uninfected roots. Naturally occurring *Armillaria* had invaded them while buried. The results indicate that *Armillaria* can survive for some time without the pseudosclerotium, which supports recent work abroad suggesting that pseudosclerotium is not required for protection.

Electron microscope studies of the effects of various fixing solutions on tobacco mosaic virus (TMV) particles. DESJARDINS, P. R., J. M. WALLACE, AND E. S. H. WOLLMAN. Successful identification of virus particles in ultrathin sections of infected tissues depends on a tissue fixation that maintains the integrity of the particles and the organization of the cell. Studies were made of the effects of some commonly used fixatives on TMV particles. Disks (6.5 mm) of TMV-infected Turkish tobacco leaves were treated for the recommended times in various fixatives. After washing, the leaf-disk samples were homogenized at pH 6, 7, and 8 and sprayed onto specimen screens. The number of particles extracted from treated tissue differed between fixatives, from practically none to as many as found in extracts from unfixed disks. In general, more particles were observed in the pH 8 homogenates. Purified virus was exposed to the same fixatives 1) after having been sprayed onto specimen screens and 2) prior to deposition on glass slides for the pseudo-replica specimen preparation technique. The main effect of the fixatives on purified virus was particle aggregation. The virus extracted from tissue and also the purified virus retained some infectivity after treatment with certain fixatives.

Downward translocation of streptomycin in Coleus sp. DOWLER, W. M., AND R. N. GOODMAN. A study of the movement of antibiotics compared upper- and lower-leaf surface applications to *Coleus* sp. About 0.5 ml of 1000 mcg/ml streptomycin solution containing 1% glycerin was brushed on proximal and distal leaf-halves that were separated by a 5-mm lanolin barrier. The antibiotic applied to either proximal or distal portions of leaves was detected after 24 hours in the untreated portion, and in stem disks taken about 5 in. below the treated leaves. However, no activity was determined in the roots. The absorption was greater where the antibiotic was applied to the lower surfaces, whereas translocation appeared to be greater from distal applications to either upper or lower surfaces. A leaf-disk assay was found to agree proportionately with the bioassay of leaf juice using a cylinder-plate method with *Bacillus subtilis* (ATCC 6633) as test organism.

Physical and chemical methods of reducing Phomopsis canker infection in Kadota fig trees. ENGLISH, HARLEY. Although *Phomopsis cinerescens* infects Kadota fig trees largely through pruning wounds, spray applications of organic or inorganic fungicides immediately before and/or after pruning have been of little value in control. Neither a standard siliceous-base wound dressing (Tree Seal) nor a fungicidal wax emulsion (Vanseal Wax plus Vancide 51) was effective in reducing infection when painted on fresh wounds. Covering the branch stubs after pruning with firmly secured plastic caps resulted in a 60% reduction in disease incidence, and painting the wounds with either Bordeaux paste or a 1% solution of Tag Fungicide (phenylmercuric acetate) before affixing the plastic reduced the incidence by about 76%. Similar control was obtained with Tag Fungicide alone at a concentration of 4%. When a synthetic latex material (Rhoplex AC-33) that dries to an elastic film was combined with Coromerc (*N*-phenylmercuriethylenediamine) and painted on pruning wounds, disease was reduced 75%. Much poorer control was obtained when either Coromerc or Rhoplex AC-33 was used alone. Another means of reducing the amount of canker is by pruning late in the dormant season, as shown by the fact that, during a 3-year period, trees pruned during February, March, and April developed 58, 42, and 27% infection, respectively.

Effects of temperature, length of the flooding period, and stage of plant growth on flooding injury to alfalfa. ERWIN, D. C., AND W. F. LEHMAN. Flooding injury to alfalfa (commonly referred to as scald) did not occur in field plots in the Imperial Valley of California after 12 hours of flooding when maximum air temperatures were as high as 46°C. After 36- and 60-hour periods of flooding, injury was most severe on plots that had been clipped either 1 or 5 days before flooding. After the 36-hour flooding period only mild plant damage occurred in unclipped plots. Although on the 60-hour flooded plots, severe damage occurred in both clipped and unclipped plots, significantly less flooding injury occurred in unclipped plots. Maximum surface water temperatures in flooded plots were usually 4-5° lower than maximum air temperatures, and soil temperatures at 2-in. depth were usually 3–5° C less than the maximum water temperatures. Both controlled greenhouse tests and field data showed that incidence of flooding injury increased with increase of temperature. The intensity of flooding injury of alfalfa is affected by 3 factors: 1) length of time the soil is flooded, 2) temperature of the soil and surface water, and 3) stage of growth of the plant following clipping.

Metabolic aspects of the self-inhibition of germination of stem rust uredospores. FARKAS, G. L., AND G. A. LEDINGHAM. The development of self-inhibition of germination in stem rust uredospores floated "en masse" on phosphate buffer at pH 6 is associated with a steady decline in their O_2 uptake. Compounds that are able to overcome self-inhibition of spore germination (coumarin, 2,4-DNP, menadione) also remove the inhibition of O_2 consumption. The beneficial effect of CO_2 on the removal of self-inhibition can be partly substituted by the administration of Krebs-cycle acids or ATP, in a CO_2-free atmosphere. The inhibition of germination apparently affects the respiratory metabolism. The decline of the respiratory rate of spores during storage parallels the decline of their ability to form the volatile inhibitor. The development of the inhibitor is apparently dependent on energy-yielding processes, because such uncouplers as 2,4-DNP inhibit its formation.

Aseptic culture of the burrowing nematode, Radopholus similis (Cobb) Thorne on excised okra root tissues. FEDER,

WILLIAM A. The burrowing nematode, *Radopholus similis* (Cobb) Thorne, was successfully cultured in a variety of glass containers under aseptic conditions on excised okra root tissues growing on modified White (1943) culture medium. Ten vigorous, mature females were placed in a sterile centrifuge tube, soaked 2 minutes in 1:1000 mercuric chloride, centrifuged, and given 2 5-minute washings with sterile double-distilled water, after which the water was poured off and the nematodes picked from the tube aseptically with a small piece of sterile cellulose sponge. The sponge containing the nematodes was placed on the agar next to the growing root culture, and the culture plus nematodes was then incubated in the dark at 27°C. Infection of the roots took place, and as many as 2000 *R. similis* individuals in various stages of development were recovered from 58 mg of roots after 1 month. The use of small cold-cream jars with plastic screw caps, or medicine bottles with cotton plugs, reduced contamination to a minimum without severely restricting growth of the okra roots or multiplication of the nematodes.

Burrowing nematode population sampling as affected by a number of variables. FELDMESSER, JULIUS. Nematode counts were made from a series of citrus feeder root samples collected in the standard manner at weekly intervals for 1 year and for 8 months, respectively, in 2 citrus groves infected with *Radopholus similis*, the burrowing nematode, to study interrelations of variables such as relative soil moisture values, air temperature fluctuations, numbers of nematodes per gram of root from inside roots and from root surfaces, and nematodes from sand within 1 in. of the root. Correlations and analyses of variance indicate that relative soil moisture has practically no influence on numbers of nematodes inside, on, or within 1 in. of, the root. Significantly more variation exists among sampling dates than among samples taken at the same date in terms of either total numbers of nematodes per sample or numbers of nematodes per gram of root. Temperature is highly correlated negatively with the number of nematodes found: the higher the temperature, the fewer the nematodes found. Correlation between average numbers of nematodes per gram of root within roots and average numbers of nematodes on root surfaces was significant at the 1% level.

Cross-protection tests with 3 strains of the aster yellows virus in host plants and in the aster leafhopper. FREITAG, J. H. Three aster yellows virus strains isolated in California have been designated as severe, dwarf, and Tulelake strains. They differ in symptoms produced on *Nicotiana rustica* and *Plantago major*. Plants inoculated with dwarf before the severe or Tulelake strain developed predominantly dwarf symptoms; plants inoculated with Tulelake before the dwarf or severe strain developed only initial Tulelake symptoms, and then recovered, although some plants later developed greatly delayed symptoms of the challenging strain. Individual leafhoppers fed 2 weeks on dwarf and then 2 weeks on severe usually (36/38) transmitted only the first strain on which they fed. Likewise, leafhoppers fed 2 days on severe and dwarf, alternately for 20 days, usually (38/41) transmitted only one or the other. In contrast, leafhoppers fed 2 weeks on Tulelake and then 2 weeks on dwarf all transmitted only Tulelake at first. Later, 16 of 24 changed abruptly, transmitting only dwarf though the other 8 continued to transmit only Tulelake. The results indicated similar interactions of the virus strains in both insect and plant hosts, depending on sequence and combination of strains used.

Influence of soil temperature on Verticillium wilt of cotton. GARBER, R. H., AND P. M. HALISKY. A study was made of the influence of soil temperature on Verticillium wilt of cotton. Constant soil temperatures were maintained in each of 4 tanks at 15, 20, 25, and 30°C. Delta-Pine 15, a wilt-susceptible variety of cotton, was inoculated with *Verticillium albo-atrum* R. & B. and grown in replicated trials at these temperatures. The experimental data obtained from 4 consecutive trials indicate that the most favorable soil temperatures for disease expression in this variety were 20 and 25°C. The average infection percentages obtained at 15, 20, 25, and 30°C were, respectively, 58, 75, 85, and 59%. No wilt symptoms developed at a constant soil temperature of 35°C introduced into this series. Growth studies were continued with the fungus isolate on PDA at constant-incubation temperatures ranging from 12 to 39°C at 3°C intervals. The largest colony diameters were observed at 18, 21, and 24°C, whereas no measurable growth occurred at 33, 36, and 39°C. The apparent lack of fungus growth above 30°C probably accounts in part for the lack of symptom expression observed in the 35°C constant-soil-temperature tank in this study.

Cucurbit virus diseases in the Imperial Valley of California. GROGAN, R. G., D. H. HALL, K. A. KIMBLE, AND R. A. KORTSEN. The most prevalent virus disease of cantaloup and squash in the Imperial Valley of California in the 1957 and 1958 seasons was watermelon mosaic (melon mosaic virus): in 77% of all virus-infected samples tested, either alone or with cucumber mosaic (cucumber virus 1). The latter virus was present in 30% of the samples, either alone or with melon mosaic virus. Only melon mosaic virus was collected in virus-infected watermelon plants. Squash mosaic (cucurbit ring mosaic virus) was extremely scarce, occurring in less than 1% of the samples. The cucurbit ring mosaic strain of squash mosaic virus was recovered from 23 of 8332 plants (0.3%) in greenhouse indices of commercial seed samples of 12 varieties of *Cucumis melo*. Seed from squash or melon varieties mechanically inoculated in the seedling stage with either watermelon mosaic virus or squash mosaic virus transmitted only squash mosaic. The percentage transmission by the different varieties and species varied from 0.0% to 25.0%. Squash mosaic virus is each year introduced into Imperial Valley cucurbit plantings in infected seed, but it remains relatively unimportant because of a lack of Diabrotica beetle vectors.

Influence of soil types on the response of ponderosa pine to atmospheric fluorides. HARRISON, WILLIAM C., AND J. WALTER HENDRIX. About 160 3-year-old *Pinus ponderosa* Dougl. seedlings in each of 8 soil series were exposed throughout the summer of 1957 to a fluoride-polluted atmosphere near Mead, Washington. The reaction of the various plants to atmospheric fluorides was determined periodically during the growing season by ascertaining 1) the % of needles showing fluoride scorch, 2) the % of total needle length scorched, and 3) the injury index (the square root of the product of items 1 and 2). A wide range in fluoride sensitivity was found among plants grown in the various soils, regardless of the basis on which plant reaction was measured. The range in reaction is exemplified by the fact that plants grown in Garrison loam, Clayton fine sandy loam, Caldwell clay loam, coarse river sand, Palouse silt loam, Ritzville silt loam, Hezel loamy fine sand, and Wenatchee loam had, respectively, injury indexes of 39, 29, 23, 22, 28, 39, 33, and 34. The results suggest that soils of medium porosity give rise to more sensitive plants, whereas plants grown in soils of extremely small or large particles tend to be more resistant.

Transmission of fanleaf virus by Xiphinema index Thorne & Allen. HEWITT, WM. B., D. J. RASKI, AND A. C. GOHEEN. Healthy grape growing with a fanleaf-diseased grape in the same clay pot developed fanleaf within 10 months of being planted and infested with 10 *Xiphinema index* adults from the root zone of healthy grape. Likewise, fanleaf developed in healthy grape growing with fanleaf-diseased grape in soil infested with *X. index* from

the root zone of fig. The following control plants of grape remained healthy: grape planted alone, grape in soil infested at transplanting time with 10 *X. index* from the root zone of healthy grape, grape in soil infested with *X. index* from fig, and healthy grape growing without infestation in a clay pot with a fanleaf-diseased grape. In all infested pots, populations of *X. index* increased by 750–39,500. In another experiment, healthy grape growing in pasteurized soil in a nursery container with a fanleaf-diseased grape remained healthy for 3 years.

Hot-water inactivation of the cucumber anthracnose organism within the seed. Horn, N. L., W. F. Wilson, and M. Giamalva. Cucumber seeds artificially inoculated by injecting fruit with a syringe containing spore suspensions of *Colletotrichum lagenarium* thereby became infected internally. Samples of these seeds were hot-water-treated at 50° and 60°C for 15 and 20 minutes, respectively, soaked in sodium hypochlorite, and plated on potato-dextrose agar. Of a total of 368 heat-treated seeds, none yielded growth of the fungus on the agar, whereas 230 seeds from the same lot soaked in sodium hypochlorite with previous hot-water treatment produced 22 colonies of *C. lagenarium* when seeded on agar. Germination of seeds treated 20 minutes at 58°C or lower was not reduced, but higher temperatures for 20 minutes did reduce germination.

The determination of pathogenic races of Fusarium oxysporum f. lini. Houston, Byron R., P. F. Knowles, and L. J. Ashworth. Sixty isolates of *Fusarium oxysporum* f. *lini* (Bolley) Snyder & Hansen were tested to determine variability of pathogenicity on 6 pure lines of flax derived from single plant selections from the 4 varieties Punjab 53, Punjab 47 (C.I. 1115), Dakota (C.I. 1071), and Argentine (C.I. 463). Greenhouse inoculation techniques previously described were used. The Fusarium isolates were all obtained from the invaded vascular tissue of plants at a level 2–3 in. above the soil line. Isolates were purified and maintained by single-sporing. Eleven isolates were obtained from plants from the Fargo (North Dakota) wilt plot, 11 from the plot at St. Paul, Minnesota, and the remainder from California. Five distinct pathogenic races of the fungus were determined by differential pathogenicity on the 6 hosts. All 5 races were found in the collections from each of the 3 states represented. Three of the 5 races could be separated on the basis of the presence or absence of 1 or more of the 3 genes (A, B, and C) for resistance previously reported. The complementary action of these 3 genes in the dominant is necessary for resistance to certain races.

Reduction in longevity of leafhoppers carrying peach yellow leaf roll virus. Jensen, D. D. Nymphs of *Colladonus montanus* Van Duzee were allowed feeding periods of 3–17 days on celery infected with peach yellow leaf roll virus. After completion of the virus incubation period in the leafhoppers (about 30 days), adults were singly caged on healthy celery plants and daily transferred to new plants until the insects died. The longevity of the leafhoppers was measured from the first day on the test plants. In 7 experiments involving 60 individuals, 39 of the 60 leafhoppers transmitted virus. The percentage of insects proving to be infective was usually proportional to the access time on the inoculum plant. Thus, only 27% became infective from 3 days on the diseased plant, but 90% acquired virus in 16 days. The longevity of insects that transmitted virus was 6–79 days, averaging 22. The non-transmitters lived 8–132 days, averaging 55. The evidence for the difference in longevity is considered to be highly significant statistically ($t = 4.17$, $p = <.01$). One hypothesis suggested by these results is that peach yellow leaf roll virus may be pathological in the insect vector as well as in the plant host.

Cronartium fusiform extent below slash pine branch cankers. Jewell, F. F. Microscopic examination of stained sections from cankers 2–3 in. in diameter and 3–7 in. in length revealed *C. fusiforme* mycelia in all swollen tissues. None were found over ½ in. beyond the margin of the canker, whether the margin was entire or consisted of longitudinal ridges extending downward from the main swelling. It is inferred that slash pine branches having rust cankers of this type can be pruned to prevent stem invasion by the fungus if the proximal margin is over ½ in. from the stem.

Factors influencing the isolation of certain soil-borne plant pathogens from soil. Kendrick, J. B., Jr., and A. R. Jackson. The presence of certain soil-borne plant pathogens is difficult to detect by soil-dilution techniques. Plating corn seed on water agar after incubation in soil has proved successful in trapping *Pythium, Rhizoctonia, Fusarium,* and *Sclerotium* spp., as well as certain other fungi. Soil moisture, temperature, and incubation period, however, vitally influence the kinds and per cent recoveries of fungi. For instance, *Pythium* and *Rhizoctonia* recoveries were greatest at medium to high moisture levels, whereas *Fusarium* was less affected by moisture, except for a decrease at the highest level. *Pythium* and *Fusarium* were favored by temperatures less than 33°C, whereas *Rhizoctonia* recovery was high even at 33°. By selecting appropriate combinations of moisture, temperature, and incubation period, specific fungi were readily detected, although not quantitatively. A more reproducible technique was developed with cornmeal agar suspension containing a measured quantity of soil. Agar disks of the suspension were transferred to water agar and incubated 10–14 days at 24°C. The kinds of fungi recovered by this technique were the same as with the corn seed "trap." In addition, a consistent relationship was noted between the amount of soil per agar disk and the percentage recovery.

Metabolic studies with the fungal toxin victorin. Krupka, Lawrence R. Previous data have shown that victorin, the toxin produced by *Helminthosporium victoriae* M. & M., causes a 3–5-fold increase in oxygen consumption of oat tissues susceptible to *H. victoriae*, but has no such effect on resistant varieties. Studies of terminal oxidase activity in victorin-treated tissues indicated that ascorbic acid oxidase activity was 2–4-fold as great in susceptible oat homogenates as in those from resistant or untreated plants. A similar increased ascorbic acid oxidase activity was found in susceptible oats inoculated with the fungus. Spectrophotometric determinations, using 2,6-dichlorophenolindophenol, showed 3-fold less ascorbic acid in toxin-treated susceptible tissue than in untreated controls. Further tests revealed that 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, had no effect on susceptible oat tissue pretreated 4 hours with toxin, and that sodium fluoride, which inhibits glycolysis, canceled the respiratory increase induced by victorin. Since healthy susceptible and resistant tissues reacted similarly to iodoacetate inhibition of glycolysis, there was no evidence that a hexose-monophosphate shunt was a factor in varietal resistance.

A unique high-pressure field-plot sprayer. Lane, Geo. H. A tractor-drawn power sprayer has been devised that provides high-pressure, mechanically uniform application of fungicides to small plots of row crops. It is unique in that the sprayer may be cleaned and the fungicide changed between plots during a single passage through the field. It was developed over a period of several years of use in spraying onion plots for Purple Blotch control. Reduction of experimental error through uniformity in the application of spray materials is a highly desirable feature in field trials. In a replicated trial on onions in 1957, 6 fungicides were compared to an untreated control. Estimates of leaf infection at harvest showed marked superior-

ity of certain fungicides. Bulb infection after a 4-month storage period likewise showed statistically significant differences. The Coefficients of Variability of these 2 sets of data are, respectively, about 10% and 17%. The fair degree of uniformity in the data reflects satisfactory uniformity in the spray application.

A serious disease of Melilotus indica associated with soils infected with Pythium. LINDBERG, G. D. Yields of *Melilotus indica*, grown as a green manure crop with sugarcane, have been reduced several hundred per cent in soils highly infested with Pythium. Two phases of the disease were readily apparent: 1) poor stands from damping-off by Pythium, and 2) severe stunting, yellowish discoloration, and premature loss of leaves in plants surviving the seedling stage. Damping-off due to Pythium, tentatively identified as *P. ultimum*, was demonstrated in the greenhouse by inoculating sterile soil with a suspension of the fungus just before planting. The incidence of damping-off was usually above 90%. A disease similar to the stunting and yellowing described here has been reproduced in the greenhouse by boring several holes in the soil around 2-week-old sour clover plants and filling the holes with a suspension of the Pythium. Within 3 weeks of inoculation the plants showed signs of stunting and discoloration. Control plants treated in a manner identical to those above, except for adding the Pythium, remained unaffected.

Antobiotics against plant disease. V. Activity of Cinnamycin against selected microorganisms. LINDENFELSER, LLOYD A., THOMAS G. PRIDHAM, AND CAROLYN E. KEMP. Cinnamycin, a polypeptide antibiotic produced by *Streptomyces cinnamomeus* f. *cinnamomeus* NRRL B-1285, has been shown to be chemically and microbiologically related to the antibiotic duramycin. Since duramycin has demonstrated antagonism against the agents of certain plant diseases, the activity of cinnamycin was reassessed in the laboratory against a wide variety of bacteria and molds, with particular emphasis on phytopathogens. Results of the laboratory studies are presented. Cinnamycin is generally ineffective against gram-negative bacteria and gram-positive cocci. Most gram-positive rods are inhibited by concentrations of 0.02–100 μg/ml. Plant pathogens inhibited by cinnamycin at concentrations under 100 μg/ml are *Bacterium cattleyae, Corynebacterium fascians, Pseudomonas phaseolicola, Xanthomonas phaseoli, Botrytis allii, Cercospora musae, Claviceps purpureae, Diplodia zeae, Helminthosporium* sp., *Rhizoctonia solani*, and *Syzygites megalocarpus* (*Sporodinia grandis*). Cinnamycin is highly bactericidal in that cells from 12 of 17 bacteria exposed to cinnamycin failed to initiate growth on transfer to antibiotic-free media.

Streptomyces spp. as a cause of natural soil fungitoxicity. LOCKWOOD, JOHN L. Mycelium of various fungi was lysed 1-2 weeks after agar cultures were covered with natural or organic soils. Mycelium of most fungi was completely destroyed, whether growing on media rich in nutrients or on deficient media. In general, mycelium of soil-inhabiting fungi lysed as readily as mycelium of other fungi. Natural soils destroyed mycelium from cultures killed by propylene oxide vapor, and inhibited germination of spores on agar. Soil sterilized by heat or propylene oxide vapor had no fungitoxic effects. Sterilized soils inoculated with isolates of *Streptomyces* spp. lytic to mycelium in culture showed lytic and inhibitory effects similar to those of natural soil. The intensity of lytic action by soils inoculated with isolates of *Streptomyces* spp. was correlated with lytic intensity of the isolate in culture. Lysis of living and killed mycelium in agar cultures by isolates of *Streptomyces* spp. does not appear to be related.

Mechanism of accumulation of Meloidogyne hapla around roots of tomato seedlings. LOWNSBERY, B. F., AND D. R. VIGLIERCHIO. In his study of nematode attraction, Wieser (1955) covered the bottom of a Syracuse dish with wet sand, placed egg masses of *M. hapla* in the center of the dish, with a germinating tomato seedling 5 mm away, and noted accumulation of larvae of *M. hapla* on the seedling side of the dish after 24 hours. The authors observe similar accumulation when free larvae are substituted for the egg masses. This substitution, accomplished by placing the larvae on sand in a glass cone suspended with its tip at the center of the dish, obviates a demonstrated hatching effect of the tomato seedling. A statistically significant accumulation of larvae on the seedling side of the dish also results when a dialytic membrane is placed between the tip of the glass cone and the seedling. This indicates that the larval accumulation is, in part, a response to a dializable agent, or agents, effective at a distance from the root surface.

Eradicative fungicides for control of gladiolus botrytis. MAGIE, R. O. Heavy production of Botrytis conidia on freeze-damaged gladiolus leaves and rotted necks prevented control of flower bud rot in spite of daily spraying with zineb. Conidial production on gray-molded tissue was only 7–17% of that on control leaves after heavy spraying with a 2.4% methyl mercury 8-hydroxyquinolinate (Ortho LM Seed Protectant), 1-2 qt. per 100 gal.; CM-19, water-soluble phenolic complex 28.5% (19% substituted halogenated phenols), 1 qt. per 100; 7.5% tris(2-hydroxyethyl) (phenylmercuri) ammonium lactate (Puratized Agricultural Spray), 1 qt./100; and 60% 1,2-bis(ethylsulfonyl)-1,2-dichloroethylene (du Pont Experimental Fungicide) 2 lbs./100 gal. Maneb 2/100 was less effective, because of leaching rain. Over 2 in. leached the sprayed leaves 1–2 days after each application. Effectiveness of du Pont Experimental Fungicide and of Ortho LM Seed Protectant increased in spite of drenching rainfall. Thiram, zineb, and captan at 2/100 were ineffective. Degree of conidial suppression tended to increase as spray applications were repeated. Germination of conidia produced on sprayed tissue was unaffected except that 5–20% of those on leaves sprayed with CM-19 and Ortho LM were aberrant or dead.

Pathological disturbances caused by Heterodera trifolii in susceptible and resistant plants. MANKAU, R. A study was made of the parasitic relationship of the clover cyst nematode to a favorable host, Ladino clover, and to less favorable hosts and resistant plants. The syncytium that develops in the stele adjacent to the head of the nematode is formed by the coalescing of adjoining cells, producing a continuous, multinucleate protoplast. It increases in size by inclusion of cells at its advancing margins. Neither conspicuous hypertrophy of cells nor nuclear division was observed. Small, tubular structures were discovered within the syncytium, close to and sometimes attached to the nematode's stylet, that are evidently related in some way to the feeding process. Highly variable development is made in pea, in which cells often react necrotically to the feeding of the nematode or its salivary secretions. Red clover is generally resistant, but occasional females develop normally. The nematode was able to develop to maturity, although usually stunted, in individual plants of other resistant crops. Regardless of host, size of females at maturity is closely related to size of syncytia, and the rate of nematode development is dependent upon rate of development of the syncytium.

Isolation for controlling internal cork of sweet potato. MARTIN, W. J., AND E. J. KANTACK. Experiments in Louisiana over a 4-year period have shown that there was relatively little spread of internal cork disease to sweet potato plantings 100 yards or more from diseased plantings. Incidence of the disease in cork-free plantings adjacent to, or short distances from, diseased plantings varied from year to year and locality to locality, ranging from less than 5% to as much as 70% of the roots affected at harvest, and

generally much higher than in isolated plantings. From 6 isolated plantings made in 1957 the mean percentages of affected roots were 1.3 at harvest and 6.9 after 4 months in storage. Plots in each of the 6 plantings in 1957 were dusted at weekly intervals during the growing season with a combination of malathion (*S*-(1,2-dicarbethoxyethyl)O,O-dimethyldithiophosphate) and DDT dust. After storage, the mean percentages of cork-affected roots from the dusted and untreated plots were, respectively, 6.3 and 7.5.

Inactivation of Semesan in soil by fungi. MUNNECKE, DONALD E., AND R. A. SOLBERG. Semesan (30% 2-chloro-4-(hydroxymercuri)phenol), applied as an aqueous suspension to soil in cotton-stoppered flasks, is inactive after 2–3 weeks in nonsterile soil but still active after 2 months in steamed soil. The inactivation is biological rather than chemical. Fungi and bacteria increased in Semesan-treated soil as the fungicidal activity decreased. Organisms isolated from Semesan-treated soil were tested for their ability to grow on potato-dextrose agar containing 0.1, 1, or 10 ppm Semesan. Several isolates of Penicillium, Aspergillus, and Trichoderma were tolerant of Semesan in agar. Flasks of soil were inoculated with spore suspensions of these fungi and incubated 1 week, and suspensions of Semesan were added. The fungicide was rapidly inactivated in soils inoculated with 2 isolates of *Penicillium* sp. and 1 of *Trichoderma viride* Pers. ex Fr. Rate of inactivation was greater in those soils than in unsteamed soil for the first 5 days, after which the 2 rates were equal.

The fungistatic effect of ionizing radiation on Botrytis cinerea in pure culture and in table grapes. NELSON, KLAYTON E., AND WARREN E. EUKEL. Mycelial transfers of *B. cinerea* on grape juice agar were irradiated with different dosages of ionizing radiation (electrons), then held at 36–38°F. A dosage of 1×10^5 rep delayed growth 5–7 days; 2×10^5 delayed growth 10–14 days; 4×10^5 resulted in slight growth, which stopped; and 8×10^5 resulted in no subsequent growth of the fungus. Cultures irradiated with 4×10^5 rep made no growth after transfer to unirradiated media. The rate of spread of botrytis rot among grape berries was markedly reduced with dosages up to 2×10^5 rep. No injurious effects were observed on fresh-harvested grapes irradiated with 2×10^5 rep.

Influence of gibberellic acid on susceptibility of celery to early and late blight. NELSON, RAY. Celery plants sprayed 3–5 weeks before normal field maturity with solution containing 20–50 ppm gibberellic acid appeared more susceptible to early blight (*Cercospora apii* Fres.), as indicated by significant increases in blight ratings over those of controls. Copper or carbamate sprays or dusts failed to protect against infection by *Cercospora apii* if plants had been sprayed with gibberellic acid before inoculation. Plants sprayed with 50 ppm gibberellic acid 3 weeks before inoculation with *Septoria apii-graveolentis* Dorogin developed significantly more lesions than comparable inoculated plants sprayed with water.

Symptoms of some fruit tree virus diseases in strawberry. NYLAND, GEORGE, AND DAVID ENGELBRECHT. Apple, apricot, peach, plum, and sweet and sour cherry shoots with, respectively, symptoms of apple mosaic, apricot ring pox, yellow bud mosaic and ringspot, line pattern and white spot, sweet cherry rugose mosaic, and sour cherry yellows were grafted to plants of the strawberry varieties Shasta and Lassen and to *Fragaria vesca*. Bottle grafts and leaf grafts were used. Symptoms in strawberry occurred in all cases and were very distinctive in several. *F. vesca* showed severe vein clearing and rugosity of leaves when grafted to apple showing symptoms of mosaic. Lassen showed leaf mottling and vein necrosis from an apricot ring pox source. Shasta showed leaf cupping and spot mottle from a line pattern in Shiro plum. Rugose mosaic from cherry and almond produced chlorotic blotches and leaf distortion on Lassen, Shasta, and *F. vesca*. Ringspot-infected peach produced mottling, vein clearing, downward curling of leaves, and short petioles in the strawberries. Shasta inoculated with the sour cherry yellows virus developed chlorotic rings and yellow mottle. Transmissions from strawberry to strawberry also were obtained with these cultures.

The influence of emanations from fruits of Prunus species on spore germination of the brown-rot organisms. OGAWA, JOSEPH M. Susceptibility of fruits or Prunus species to brown-rot organisms is known to increase with fruit maturation. This behavior has been attributed to skin thickness or chemical constituents in the fruits or in the hypodermis of the fruits. Dusting green and ripe peach fruits with spores of *Sclerotinia (Monolinia) fructicola* and incubating the inoculated fruits in a saturated atmosphere resulted in less spore germination and shorter germ tubes on the surface of green fruits than on ripe fruits. Gaseous emanations from fruits of certain Prunus species markedly affected the germination of spores of *Sclerotinia laxa* and *S. fructicola*. Bioassays were made by exposing spore suspensions in micro-beakers to the emanations from green and ripe fruits. Green fruits (peach) produced inhibitory effects on germination of spores of *S. fructicola*, whereas ripe fruits (peach, apricot, and cherry) produced stimulatory effects on spore germination of both *S. laxa* and *S. fructicola*. The maximum increase in spore germination was from 8 per cent, in the check, to 97 per cent, in those exposed to ripe peach emanations, whereas the maximum decrease in spore germination was 69 per cent, in the check, to 15 per cent, in those exposed to the green peach emanations.

Studies on a soil-borne potato virus disease in California. OSWALD, JOHN W., AND TULLY BOWMAN. Potato tubers, var. Kennebec, with shallow necrotic rings (5–10 mm in diameter) just below the skin and internal necrotic flecks were observed in local areas of 4 fields in the Salinas Valley. Mechanical inoculation with sap from the necrotic rings caused a necrotic mottle to develop in *Nicotiana tabacum*, vars. Turkish and Yellow Burley. Necrotic rings on the leaves, severe stem necrosis, and stunting characterized the disease in tobacco. Seventeen of 142 tobacco plants (12%) developed identical symptoms when grown in soil from the diseased areas; 56% of diseased tubers produced at least 1 plant with distortion, yellow mottle, and stem necrosis. Only 14% of the total stems exhibited this symptom, 44% of the progeny tubers from diseased plants showed rings and internal flecking, similar to the field disease. Constantly associated with the disease are straight, virus-like rods with modal lengths of 106 mμ and 195 mμ by about 20 mμ in width. Preliminary serological tests with anti-serum from Holland to the tobacco rattle virus indicate a relationship between it and the Salinas virus. The disease appears to be viral in nature, and similar to the potato stem mottle and tobacco rattle virus disease described in Europe.

An effect of substrate on spore form in Phomopsis. PARMETER, J. R., JR. Examination of a diseased *Daubentonia* specimen showed typical *Phomopsis* pycnidia on the bark-covered portions of the stem. On decorticated areas, pycnidia containing only scolecospores were observed. Since identification of *Phomopsis* may depend on the presence of both ovoid alpha and acicular beta spores, consistent absence of alpha spores on some plant substrates could lead to incorrect diagnoses. To test the constancy of spore form on different plant substrates, sections of *Daubentonia* stem, with and without bark, were sterilized in propylene oxide and placed on water agar in Petri dishes. These were inoculated with a spore suspension derived from a culture that originated from a single alpha spore. After incubation for 20 days at room temperature, spores began to ooze from the developing pycnidia. Examination of the

spore masses from individual pycnidia showed that only beta spores were produced in pycnidia on decorticated wood, whereas both alpha and beta spores were produced in pycnidia on bark. These observations emphasize the importance of testing fungus isolations on a variety of natural substrates before making determinations.

A disease of Kentucky blue grass incited by certain spiral nematodes. PERRY, VERNON G. Large populations of plant-parasitic nematodes are found in Kentucky blue grass (*Poa pratensis* L.) turf in central and southern Wisconsin. Certain species of spiral nematodes (*Helicotylenchus*) of which descriptions are being prepared for publication, are most common and abundant. Pathogenicity to blue grass was established by greenhouse inoculation experiments, field control experiments, and pathological histology studies. Affected plants are stunted, their roots are discolored brown, the root cortex apparently is sloughed prematurely, and the plants enter what has been termed "summer dormancy"—usually in June in Wisconsin. Through applications of 2 nematocides the stunting, root discoloration, and summer dormancy symptoms were corrected to such an extent that weeds, clover, and Bermuda grass were largely crowded out by the Kentucky blue grass. The spiral nematodes were repeatedly observed in a feeding position, with their heads in root cortical and phloem tissues of greenhouse-grown inoculated plants and field-grown plants.

Dips and drenches for the control of Fusarium stem rot of carnations. PETERSEN, L. J., AND RALPH BAKER. The conidia of *Fusarium roseum* f. *cerealis* (causal agent of Fusarium stem rot) were found on carnation cuttings used in propagation. Infested cuttings were dipped 10 minutes in various solutions and suspensions of fungicides in attempts to eradicate this inoculum from cuttings. Results indicated that Panodrench 4 (3.0 ppm cyano(methylmercuri guanidine), Panogen experimental material No. 13849 (3.0 ppm active ingredient), and ferbam (1000 ppm ferric dimethyldithiocarbamate) were effective in control. In a representative test, 27 of 36 plants were dead in the inoculated control 60 days after the propagative period, whereas 0, 2, and 4 plants were dead in plots in which cuttings had been dipped originally in Panodrench 4, Panogen No. 13849, and ferbam, respectively. At the same rates these compounds were equally effective when applied as drenches at the beginning of the propagative period. Combination dips and drenches were slightly phytotoxic, but gave better control than did dips or drench treatments alone.

The effect of light upon growth of Armillaria mellea in culture. RAABE, ROBERT D. On culture media, the growth of *Armillaria mellea* was more abundant in the dark than in the light. In an attempt to separate the effects of different areas of the spectrum, agar cultures of the fungus were grown under Cellophane of different colors. The fungus grew well in yellow light, less well in green light, still less well in red light, and poorly in blue light. Cultures grown in the dark were used as checks. Because of the optical impurity of the Cellophane as tested by a spectrophotometer, and because of the difficulty in regulating intensity, cultures of the fungus were grown under Kodak Wratten filters mounted in "B" glass. By arranging them at different distances from the light source and using neutral density filters, light intensity was so regulated that all cultures received the same amount of light. The fungus developed poorly under blue and red filters, better under a green filter, and very well under a yellow filter.

The incorporation of C^{14} from labeled organic acids into tobacco mosaic virus. SCHLEGEL, DAVID E., AND H. GÜNTER WITTMANN. Tobacco-mosaic-virus-infected tobacco leaf tissue (*Nicotiana tabacum* var. Trelease) was fed C^{14}-labeled oxalic, malic, and acetic acids, either by vacuum infiltration into inoculated leaf disks or by petiolar feeding. Feeding was done on the third day after inoculation, and the tissues were allowed to metabolize the labeled organic acids for 24 hours. The virus was purified, its protein and nucleic acid separated, and the radioactivity of each fraction determined. Only a relatively small amount of the C^{14} from the organic acids supplied was incorporated into the virus—0.5% in the case of malic and acetic acids, and 0.03% in the case of oxalic acid. The C^{14} from the organic acids was found mainly in the protein fraction; however, the label from acetic acid also appeared in small amounts in the virus nucleic acid. Paper chromatography of hydrolysates of the protein fraction showed that radioactivity was rather generally distributed in the amino acids. The very low rate of incorporation of organic acid and the general distribution of the label in the amino acids seem to indicate a nonspecific role of organic acids in virus multiplication.

Anatomy of tristeza-diseased lemon seedlings. SCHNEIDER, HENRY. In Feb., 1957, Lisbon lemon seedlings 15 cm tall were side-grafted with lime scions that were not later removed. The diseased scions were from plants that had been insect-inoculated from commercial sweet orange and from Meyer lemon. Plants were sacrificed for sectioning at 10, 15, and 27 weeks. Plants inoculated with virus from Meyer lemon developed small yellow leaves with curved midribs at 15 weeks, and their feeder roots were deteriorated at 27 weeks. They grew only half as tall as other plants in the experiment, but radial growth of the stem at the transition region was not impeded. Both virus strains caused chromatic cell formation throughout the plant in all collections. Feeder roots of plants inoculated with virus from sweet orange exhibited slight sieve-tube necrosis and slightly excess phloem in occasional roots. In contrast, feeder roots of plants inoculated with the strain from Meyer lemon exhibited extensive sieve-tube necrosis, tremendous excess phloem production, and very little secondary xylem, and sometimes vessels were gummed. Both virus strains affected lime plants to about the same degree. In lemon, unlike the condition in limes, the chromatic condition did not spread to meristems.

Rapid entry of infectious particles of southern bean mosaic virus into living cells following movement of the particles in the transpiration stream. SCHNEIDER, I. R., AND J. F. WORLEY. Infectious particles of southern bean mosaic virus moved upward in the transpiration stream through steamed regions of Pinto bean stems, entered living cells, and multiplied. A hypodermic needle was used to introduce purified virus into the hypocotyls. The virus frequently multiplied in foliage far above the point of inoculation, as indicated by the appearance of characteristic necrotic areas (sometimes within 4 days). Juice obtained from such areas was always highly infectious. Necrosis in foliage did not develop after inoculation of the hypocotyl surface. Application of steam to a portion of an internode above the inoculated area (24–48 hours before virus introduction) did not retard the upward movement of virus. Necrosis associated with virus multiplication occurred as frequently and as rapidly in plants with steamed internodes as in plants with internodes not steamed. Neither necrosis nor virus was ever detected in uninoculated plants. Introduction of the virus into an internode well above the hypocotyl also resulted in the frequent occurrence of necrosis in foliage above the inoculated area, but rarely in that below.

Vector efficiency of transmission of 2 pepper viruses as influenced by species and clone of aphid. SIMONS, JOHN N. Efficiency of transmission of southern cucumber mosaic virus in pepper was measured with a single clone of the green peach aphid, *Myzus persicae* (Sulz.), and with 3 clones of the cotton aphid, *Aphis gossypii* Glover, over a period of 3 years. Transmission of vein-banding mosaic virus in pepper was measured by 2 clones of green peach aphids and the 3 cotton aphid clones over a 2-year period.

Each clone transmitted each virus with different, but characteristic, efficiencies, which remained constant during the period of sampling. Attempts to change vector efficiency by changing the host plant of the aphid clone sometimes succeeded, but this change was not as great as would be expected if the aphid host plant were the primary reason for the variation. Clones that were poor vectors of one virus were poor vectors of the other virus.

Reaction of four Rhizoctonia solani isolates to certain chemicals. SINCLAIR, J. B. Certain chemicals, determined by greenhouse assay to be effective against damping-off of cotton seedlings (incited by *Rhizoctonia solani*), were placed in out-field tests in 1957. Significant differences in stand count were noted only in the field plot from which the culture of *R. solani*, used in the greenhouse tests, was originally isolated. Greenhouse studies were made in 1957-58 to test the extent of disease control by 2 chemical combinations (captan plus PCNB and nabam plus PCNB) against 4 isolates of *R. solani*. Greenhouse flats with sterilized field soil were infested with 10-day-old potato-sucrose broth cultures of the fungus isolates, and sowed 2 weeks later with Deltapine 15 cotton seed treated with Dow 9B in 7 replicates, 50 seeds per replicate. Untreated-inoculated and untreated-uninoculated flats served as controls. Emergence and disease counts were taken at least 2 weeks after planting, and the data were expressed in percentage of healthy plants. The experiment was repeated 3 times, with essentially the same results. Of the 4 isolates, 3 were highly pathogenic. Highly significant differences in percentage of healthy seedlings were found between the 4 isolates within both chemical treatments.

Cultural differences in isolates of Scirrhia acicola from Pinus palustris and P. taeda. SNOW, GLENN A. Because *Scirrhia acicola* has recently been prevalent on *Pinus taeda* as well as its usual host *P. palustris*, isolates from the 2 pines were compared to determine if physiological strains could be distinguished in culture. No significant differences were observed in rate of lateral growth on PDA or malt agar, in size of conidia or conidiophores, or in width of hyphae. Variations in color of mycelial mats, shape of colonies, sectoring, and abundance of conidia were as great among isolates from the same pine species as among those from different species. A mutual inhibition zone was observed between all combinations of isolates during the first month of growth. Later, the mats joined, and hyphal fusions were observed between isolates from *P. palustris* and *P. taeda*.

Hot air for the control of the ratoon stunting disease of sugarcane in Louisiana. STEIB, R. J., AND I. L. FORBES. Hot air was chosen instead of hot water to control the ratoon stunting disease after greenhouse and field tests proved it safer to use. An oven of 2200 lb. capacity was made available to the industry after it was thoroughly tested. Performance tests of 60 ovens on farms were made in 1955 and 1956 with thermocouples. Oven operation was satisfactory during the required 8-hour period. An in-going temperature of 58°C is required to give an oven air temperature of 54°C. Cane treated on various farms germinated well, and the disease was controlled to a high degree in most varieties. The level of control was not always 100%. A check of the second-year progeny (first ratoon) showed that the disease had spread very rapidly where a harvester had been used, the knives having become contaminated by the few diseased plants found in the plant cane. Plant cane found 2.1% infected showed 16% of the plants diseased in the stubble crop. From results of 2 tests using various temperature-time combinations, it appears that certain varieties may require a specific hot-air treatment for maximum disease control.

Effect of temperature on control of seed-borne teliospores of safflower rust with a volatile mercury fungicide. THOMAS, C. A. Safflower seed heavily infested artificially with teliospores of *Puccinia carthami* and slurry treated with Panogen 15 (2.2% cyano(methylmercuri)guanidine) at dosages of 1–4 oz./bu. was held 3 days in sealed storage at 5, 10, 15, 20, 25, 30, and 40°C. Treated seeds were planted in flats of moist, steamed soil, which were held 1 week at 5°C in a cold room and 4 weeks at 20°C in the greenhouse. Plants from infested but untreated seed were 100% rusted. The number of rusted seedlings produced from treated seed decreased as either storage temperature or dosage was increased. Maximum percentages (1–5%) of rusted seedlings were produced at storage temperatures of 5–15°. Less than 0.5% rusted seedlings developed from seed treated at 2–4 oz./bu. and stored at 25–40°. None of the treatments gave perfect control, affected stand, or produced visible injury.

The effect of the root-knot nematode, Meloidogyne javanica, on blackeye bean wilt. THOMASON, IVAN J. The 2 most important diseases of blackeye beans, *Vigna sinensis*, in California are Fusarium wilt and root-knot. Greenhouse tests with the 2 varieties Grant and Chino 3 have shown that wilt was more severe in plants grown in soil infested with both Fusarium and nematodes than in soil infested with the fungus alone. Grant was found resistant to some isolates of the wilt fungus, *Fusarium oxysporum* f. *tracheifilum*, when the plants were tested by dipping the roots in a spore suspension. The resistance of this variety was reduced when the roots of the plants were infected by nematodes. In 2 field tests on a sandy soil in which root-knot nematodes were controlled by soil fumigation (0.5 gal./A. 1,2-dibromo-3-chloropropane), the severity of Fusarium wilt was markedly reduced in both Grant and Chino 3. Dry bean yields were increased from 600 lb. on untreated land to 1600 lb./A. on treated soil.

Treatment of pink-root-infested soil with Vapam and Mylone. TIMS, EUGENE C. Soil from 2 sources heavily infested with the pink root fungus, *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson, as well as soil or sand artificially inoculated with the fungus, were used in greenhouse tests. Vapam (40% sodium *N*-methyldithiocarbamate dihydrate) and Mylone 85-W (3,5-dimethyltetrahydro-1,3,5-2*H*-thiadiazine-2-thione), 85% by weight, were used at rates of, respectively, 1–4 qt. and ¾–5 lb. per 100 sq. ft. Onion seedlings were used as test plants to indicate the severity of pink root. Vapam gave almost complete control of the disease in naturally infested soil at all the rates used. Mylone gave good control at the heavier rates, but at the lower rates there was some pink root development. In artificially inoculated sand or soil, the chemicals were not nearly so effective in reducing the amount of pink root. No phytotoxicity occurred if 15–21 days elapsed between applying the chemicals and planting the onion seed. Nematode populations in the natural field soil were almost completely destroyed by the heavier applications of Mylone and Vapam.

Serial dilution method for estimating disease potentials of citrus Phytophthoras in the soil. TSAO, PETER H. The conventional methods for quantitative determination of populations or disease potentials of microorganisms in the soil are not applicable to *Phytophthora citrophthora* or *P. parasitica*, incitants of gummosis, brown rot, and fibrous root rot of citrus. A serial-dilution method, using lemon fruit as host, was developed that provided means for studying distribution and factors influencing the disease potential of these fungi in the soil. A 50-cc sample of a thoroughly mixed soil was divided into 2 equal portions. One half was used undiluted; the other was diluted with sterile soil to give 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 dilutions. The comparative disease potential of *Phytophthora* spp. in a soil was estimated by means of a standardization lemon trap test (25 cc soil, 150 cc water, 2 ripe or near-ripe lemon fruits, 25°C, and 6-day duration). The tests were

made in 1-pint wide-mouthed paper cups. The disease potential index of a given soil was defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the test fruits. A soil with an index of 16 is considered to have a disease potential about 8 times that of a soil with index 2.

The pathogenicity of Hemicycliophora arenaria on citrus. VAN GUNDY, S. D. *Hemicycliophora arenaria* Raski was found parasitizing rough lemon (*Citrus limonia* Osbeck) roots in the Coachella Valley, in California. In the field the nematode was associated with a distinctive galling of the terminal and lateral root tips. Rough lemon and Homosassa sweet orange (*Citrus sinensis* Osbeck) seedlings were inoculated with 250 hand-picked nematodes and maintained in soil temperature tanks at 25° and 30°C. The better temperature for gall production and reproduction of the nematode on lemon roots after 5 months was 30°. The trees grown at 30° had 350 galls, whereas those grown at 25° had 100 galls. The total number of nematodes recovered per tree from the soil and roots was 160,000 nematodes at 30° and 10,000 nematodes at 25°. There was a 35% reduction in top growth at the higher temperature. No galling occurred on the sweet orange roots. The nematode population in each of the inoculated crocks was less than the original inocula. The formation of galls on rough lemon roots was a hyperplastic response to the feeding of the nematodes. The cells adjacent to the stylet of the nematode showed hypertrophy of the nuclei.

The prediction of widespread late blight weather from synoptic pressure patterns. WALLIN, JACK R., AND JOHN A. RILEY. Recording hygrothermographs among potato and tomato foliage were used to estimate the duration of coincident temperatures and relative humidities associated with the growth and development of the late blight pathogen, *Phytophthora infestans*. Spread of the pathogen was predictable for a 7-day period if coexistent time-temperature-relative-humidity values were: mean temperatures of 60–80°F for 10 hours or more, 54–59°F for 13 hours or more, or 45–53°F for 16 hours or more, all with relative humidities of 90%. Daily sea-level pressure maps were studied to identify patterns most indicative of these blight-favoring periods. In the North Central States, the incidence of these periods was closely related to a nearly stationary east-west front just south of the target area, accompanied by an open wave or an active low-pressure system. Air flow patterns at the surface and at about 10,000 ft. were obtained from 5-day mean-sea-level and 700-millibar pressure maps. The circulation pattern most indicative of favorable periods was a southeast flow at the surface coincident with a southwest flow at 10,000 ft. Using this pattern as a basis, objective 5-day forecasts of widespread favorable or unfavorable blight weather were generally successful.

Cross-protection studies with yellow-vein and certain other viruses of citrus. WEATHERS, L. G. To determine the possible relationship of yellow-vein to other virus diseases of citrus, West Indian lime (*Citrus aurantifolia*) seedlings infected singly with cachexia, exocortis, psorosis, tristeza, and vein-enation viruses were graft-inoculated with the yellow-vein virus. Yellow-vein symptoms developed within 3–6 weeks in all inoculated plants, irrespective of the presence of the other viruses. Yellow-vein symptoms were much more pronounced in plants containing either cachexia or vein-enation than in plants infected with yellow-vein virus alone. Conversely, the presence of psorosis slightly suppressed the severity of yellow-vein symptoms. Tristeza and exocortis viruses did not alter the expression of yellow-vein virus. Symptoms of cachexia, exocortis, psorosis, and tristeza viruses were not noticeably affected by the presence of the yellow-vein virus. Vein-enation symptoms were more prominent in the presence of yellow-vein. The failure of the viruses of cachexia, exocortis, psorosis, tristeza, or vein-enation to provide protection against infection and development of yellow-vein symptoms in the reinoculated lime plants suggests that yellow-vein virus is not related to these other 5 viruses.

Resistance to Phytophthora cinnamoni in the genus Persea. ZENTMYER, GEORGE A. Phytophthora root rot of avocado poses a major threat to the avocado industry in California. In the search for a resistant rootstock, seeds of varieties of avocado (*Persea americana*) and of other native species of *Persea* were collected in Mexico, Central America, Colombia, Ecuador, Peru, Chile, Argentina, Brazil, Venezuela, Trinidad, Puerto Rico, and Cuba. High resistance to *Phytophthora cinnamomi* was found in 5 small-fruited species of *Persea: P. Skutchii, P. caerulea, P. chrysophylla, P. Donnell-Smithii,* and *P. alba.* Moderate resistance was found in 2 *P. americana* types: the Duke variety, a Mexican seedling grown in California for many years; and a West Indian type from Colombia. Compatibility tests by E. F. Frolich and G. F. Ryan showed that the highly resistant, small-fruited types are not graft-compatible with commercial avocado varieties in California. Collections were tested by a rapid, severe method using aerated nutrient solution; and by the less severe method of planting seedlings in infested soil in greenhouse or lathhouse. Correlation was good between reaction to inoculation with *P. cinnamomi* in nutrient solution and performance in infested soil in field plantings. Further selections are being made from the Duke variety in various locations in California.

CONTROL OF FUSICOCCUM CANKER OF PEACH BY NUTRITION, DEFOLIATION, AND PROTECTIVE FUNGICIDES [1]

Robert H. Daines,[2] D. F. Cohoon,[3] Ida Leone,[4] and Eileen Brennan [4]

SUMMARY

Nodal cankers produced on peach trees by *Fusicoccum amygdali* Del. appear throughout the year, with peak occurrence in spring and fall. Field and greenhouse experiments showed that twig infections occur at unhealed bud scales, fruit and leaf scars, at the blossoms, and at points of mechanical injury to twigs, buds, leaves, and fruit. Laboratory studies indicated that resistance to the disease is related in part to the development of protective cork layers or periderm at these surfaces, and to the nutritional status of the trees. Study of the fungus in artificial media indicated the following conditions to be favorable for growth and fruiting: 80–85°F temperature, constant light, high sugar concentration, and certain organic nitrogen sources. Control may be achieved with protective materials applied at the delayed dormant stage and continuing through the spring, summer, and leaf-dropping stages. Forced defoliation with a cyanamid spray or dust shortens the period during which protective leaf drop sprays are needed and ensures periderm development. Since a periderm imparts protection to scars, the defoliant or fungicides used should not adversely affect its development. Cultural and fertilizer practices should be such as to avoid excessively high nitrogen in trees in the fall.

INTRODUCTION.—Peach canker, first observed in New Jersey by the senior author in 1934, has in recent years threatened the continued culture of most peach varieties in the southern part of the state. Among the varieties severely affected are Golden Jubilee, Eclipse, Raritan Rose, Jerseyland, Early East, Cardinal, Brackett, Constitution, Summercrest, Triogem, J. H. Hale, Belle of Georgia, Keystone, Newday, and Red Haven. Sunhigh, Redcrest, Dixired, and Coronet seem to exhibit some resistance to the disease under orchard conditions. The disease has been reported in Delaware (17), Maryland (20), New York (15), Massachusetts (1), Rhode Island, and Virginia, and abroad in France (8) and Japan (14).

The causal fungus, *Fuscicoccum amygdali* Del., was first described in 1905, by Delacroix (7), who reported that it produced cankers at the nodes of 1-year-old shoots of almond trees in southern France. The conidia were 5–7.25 × 2.5–3 μ, ovoid, often pointed at 1 end, and with 2 oil globules. Beta spores were not mentioned in this report.

From the earliest history there has been some confusion in ascribing the cause of this disease to a particular organism (2, 9, 10, 12, 17, 18, 19). In 1941 Haenseler and Daines (13) described a nodal canker disease in New Jersey that they attributed to *F. amygdali*. In their studies of the organism no beta spores were found, and spore size and description agreed well with those of Delacroix for *F. amygdali*.

Peach canker affects 1- and 2-year-old twigs, blossoms, leaves, and fruits. The most evident symptom of the disease is the presence of dark-brown cankers at the nodes of the current season's twigs (Fig. 1), which later become grayish tan. Cankers occurring at the buds (bud cankers) are usually 1–2 in. long, whereas those at the base of the current season's twigs (basal cankers) may be larger. Cankers are not as evident on 2-year-old corky bark as on younger wood. Cankers often girdle the twigs, producing wilting and eventual death of the distal portion. Where girdling is incomplete, a flattened and weakened branch results. Cankers appear throughout the year, with the greatest number in the spring and fall. Bud killing may occur at all stages throughout the fall and spring. Twig-flagging during May and June results from bud cankers from infections occurring in the fall and spring; flagging during August and into the fall results from basal cankers induced at the terminal bud scale scars during the early growing period as well as from cankers induced at the leaf scars during early fall. Cankers are often greatly enlarged by a second fungus, *Valsa leucostoma* Pers. ex Fr. (Fig. 1), during the fall and winter (5). Characteristically, the bark over these latter cankers is loosened, light tan, and often zonate. Gumming is common on all twig cankers.

Blossom blight caused by Fusicoccum appears similar to that caused by brown rot, but can be distinguished from brown rot by its failure to produce conid-

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FIG. 1–6. Fig. 1–3. Symptoms induced on peach by *Fusicoccum amygdali*.—Fig. 1. *F. amygdali* twig canker surrounded by Valsa canker.—Fig. 2. Leaf infection.—Fig. 3. Fruit infection showing erumpent pycnidia.—Fig. 4–6. Effect of nutrition on growth and fruiting of *F. amygdali*.—Fig. 4. Effect of glucose concentration. A) 0.2%. B) 0.8%. C) 3.2%. D) 6.4%.—Fig. 5. Effect of nitrogen source. A) peptone. B) glutamine. C) ammonium sulfate; note dark pigmentation. D) sodium nitrate.—Fig. 6. Effect of light. A) constant darkness. B) constant lighting.

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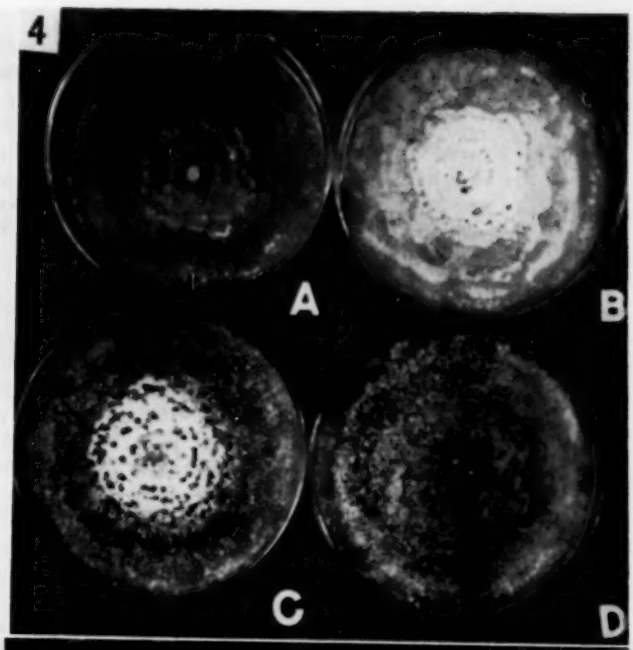
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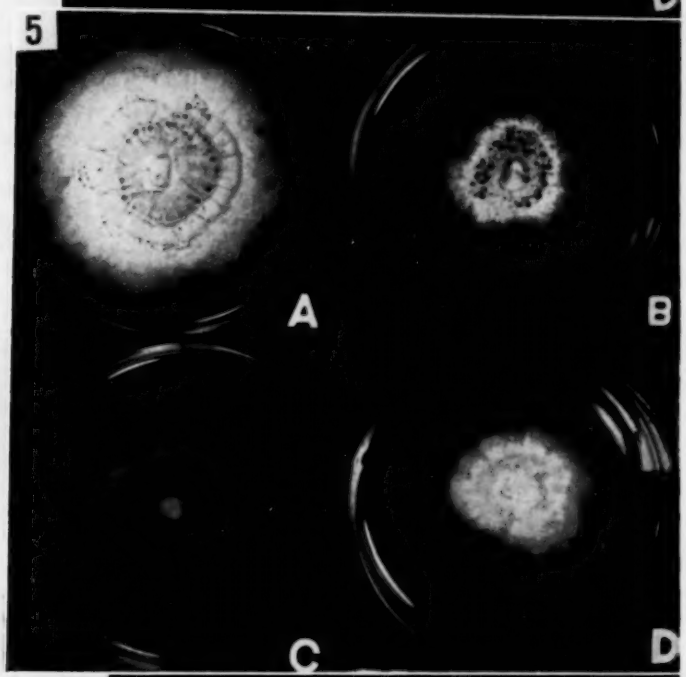
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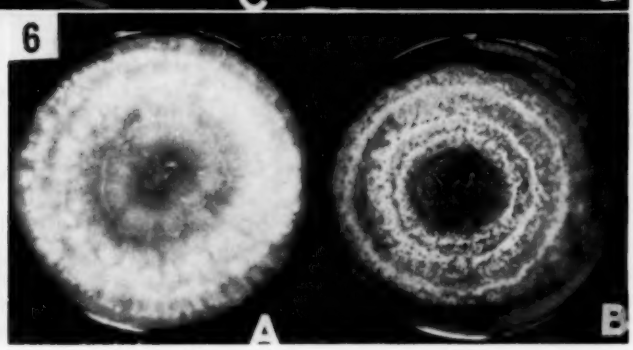
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ial tufts. Fusicoccum often invades the blossom pedicel and progresses into the twig, resulting in a typical twig canker.

Symptoms of peach canker on leaves and fruit were first observed by Daines (5), in 1952. Necrotic areas on the leaves were V-shaped, with a vein or veinlet occupying the base of the V. Although infection frequently occurred at the tips of the leaves, it could also be found elsewhere on the leaf (Fig. 2). Infected leaves usually showed a chlorotic zone in advance of the V, and premature abscission was common. Black pycnidia developed in the older portions of the lesion. These symptoms are not to be confused with lesions lacking the characteristic V-shape and pycnidia that are caused by physiogenic factors (4). The diseased area in the fruit was brown to grayish, with a somewhat darker center (Fig. 3). Black pycnidia appeared abundantly in the center of the lesion as the rot progressed. The infected fruits soon shriveled, eventually forming mummies. Although the incidence of fruit rot was usually very low, 10 per cent of the fruits in a well-dusted sulfur block of Sunhigh trees were affected in 1952. Fruit infection invariably originated at bacterial spot lesions or at insect injuries.

EXPERIMENTAL METHODS.—*F. amygdali* was grown on potato-dextrose agar for temperature studies, and on a variety of media for nutritional studies dealing with various carbohydrate and nitrogen sources. To determine the optimum temperature for growth, cultures were incubated 1 week over a temperature range of 41–99°F. In later experiments, cultures were incubated at 80°F. Observations were made on vegetative and fruiting characteristics.

Seven-day-old cultures of the fungus growing on potato-dextrose agar were used in orchard inoculations. A piece of the mycelial mat was used as inoculum, and moist absorbent cotton was wrapped above and below the inoculated site. The entire area was then enclosed with aluminum foil, for 3–7 days during warmer seasons and as long as 1 month during the winter. Natural infection was simulated by applying a heavy conidial suspension with a small camel's-hair brush. The conidia were obtained from spore tendrils oozing from cankered wood. Greenhouse inoculations were made either by spraying the entire tree with a standardized spore suspension or by applying the spore suspension directly to the infection court. After inoculation, trees were incubated 3 days at 80°F and 90–100 per cent relative humidity.

Defoliation was accomplished in the greenhouse by removing the leaf blade, leaving only the leaf petiole, which loosened and fell within 4 days. This method was chosen instead of chemical defoliation to avoid possible interference with normal healing.

In histological studies, leaf and bud scale scars were prepared for examination, either by freehand sectioning of fresh material or by microtome sectioning of embedded material. Tests for gum were made by the phloroglucinol reaction and the Maule test (16), and the presence of suberin was detected by staining with Sudan IV.

Experiments on control were conducted on blocks of 20 randomized trees by applying fungicides with a bean sprayer at the rate of 30 gal. per minute at 300 lb. pressure, using an 8-cluster nozzle broom gun. Where entire rows of trees served as a treatment, applications were made to alternate rows with a windblast speed sprayer. The fungicides used were sulfur; ziram (76% zinc dimethyldithiocarbamate); ferbam (76% ferric dimethyldithiocarbamate); thiram (65% bis(dimethylthiocarbamoyl) disulfide); captan (50% *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide); zineb (65% zinc ethylenebis[dithiocarbamate]); dichlone (50% 2,3-dichloro-1,4-naphthoquinone); tank-mixed zineb (19% nabam (disodium ethylenebis[dithiocarbamate]), zinc sulfate, and lime in a ratio of 2 qt. to 1 lb. to 1 lb.); and a tank-mixed fungicide consisting of 19% nabam, ferric sulfate, and lime (2 qt. to 10 oz. to 1 lb.). Additional fungicides used in laboratory tests were maneb (70% manganous ethylenebis [dithiocarbamate]), glyodin (34% 2-heptadecyl-2-imidazoline acetate), Dyrene (50% 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine), Omadine (50% zinc salt of 2-pyridinethione-l-oxide), Agricultural Puratized (7.5% phenylmercury triethanol ammonium lactate), Bordeaux mixture, mono-calcium arsenite, calcium cyanamid, and Copper "26" (basic copper sulfate).

EXPERIMENTAL RESULTS.—*Effect of environment on the fungus.*—1) *Temperature.*—The effect of temperature on growth was determined by incubating cultures of *F. amygdali* 1 week over a temperature range of 41–99°F. Optimum growth occurred between 80° and 85°F. Only 1 isolate showed growth at 41°F; none grew at 99°F. The effect of temperature on germination of conidia was investigated by observing the time required for germination at temperatures ranging from 30° to 100°F. The optimum temperature for germination was about 80°F: 50 per cent of the spores germinated in only 7 hours. At 100°F only 30 per cent of the spores had germinated by 30 hours. The remaining spores showed a tendency to enlarge without producing a germ tube. At 32°F, 50 per cent germination was obtained by 16 days.

2) *Nutrition.*—To provide information on cultural characteristics, plates of Czapek, Saboraud, Grosclaude (8), oat, Lima bean, bean pod, and potato-dextrose agars were inoculated with *F. amygdali* mycelium, incubated 1 week at 80°F, and placed at room temperature in intermittent light for 2 weeks more. On all media—except for oat agar, which allowed only very meager growth—white mycelial colonies showing pronounced concentric zonation covered the entire Petri dish. On Czapek and Grosclaude agars, fruiting appeared within 1 week; Saboraud agar showed fruiting after 17 days; and potato-dextrose agar showed little fruiting even after 3 weeks.

Tests were conducted to determine the effect of varying carbohydrate sources on growth of the organism. For this purpose, media were prepared in which glucose, sucrose, soluble starch, and cellulose were used as the energy source at a concentration of 0.1 per

cent by weight. Mycelial growth was similar on all carbohydrate sources except cellulose, which supported only slightly more growth than the medium lacking carbohydrate. Fruiting was generally sparse, but slightly more pycnidial development occurred on the sucrose-containing media than the glucose-containing media. No fruiting occurred on the media containing starch or cellulose. Since little variation was induced by the 2 sugars used at a 0.1 per cent concentration, glucose and sucrose were tested at concentrations of 0.2, 0.8, 3.2, 6.4, and 12.8 per cent by weight. Although mycelial growth during the first week was similar at all levels for both sugars, differences in vegetative growth and fruiting were evident by the second week. In media containing sucrose at concentrations of 6.4 per cent and above, or glucose at a concentration of 12.8 per cent, growth became rugose and viscoid and was characterized by dark-brown pigmentation. Fruiting was clearly dependent on sugar concentration (Fig. 4). At concentrations below 3.2 per cent, neither sugar produced cultures with evidence of pycnidial formation by the end of the second week. Fruiting increased as the sugar concentration was increased above 3.2 per cent. Sucrose appeared slightly more conducive to fruiting than glucose.

The foregoing media contained an inorganic nitrogen source, sodium nitrate. Experiments were conducted in which various organic as well as inorganic nitrogen sources were incorporated in quantities providing 0.04 per cent nitrogen in a basic glucose medium. Most of the nitrogen sources supported considerable vegetative growth, except for glycine, which permitted little or no growth. Fruiting was evident by the end of 1 week on the following cultures, in descending order of pycnidial abundance: peptone, glutamine, urea, asparagine, ammonium sulfate, and sodium nitrate (Fig. 5). The inorganic sources in this series were considerably less conducive to fruiting than the organic. There was no evidence of beta spore production in any of these experiments.

3) *Light.*—The effect of light on growth and fruiting was tested by incubating inoculated plates of potato-dextrose agar in constant darkness and in continuous light supplied by a 40-watt electric light bulb at a distance of 6 in. Within a week the lighted cultures showed numerous pycnidia, whereas the cultures kept in the dark remained sterile (Fig. 6). Vegetative growth was similar in both cases.

To assure abundant and rapid fruiting of *F. amygdali* on artificial media, it seems advisable to include a high sugar concentration, an organic nitrogen source of the type specified above, an 80°F temperature, and constant exposure to light.

Infection courts.—Orchard counts by Cohoon and Daines (4) indicated the appearance of cankers throughout the year, with the greatest number in the spring and fall, when opportunity was greatest for entrance through unhealed scars from removal of leaves, bud scales, blossoms, and fruit. These, as well as blossoms themselves, have been reported as suitable infection courts for the fungus (3, 4, 5, 8). In addition, dead or injured buds or bark areas have been suggested as possible infection courts (5, 8). Although Guba (10) reported that ingress occurs through leaf scars and buds, he did not mention any necessity for injuries or unhealed areas.

TABLE 1.—*Canker development on Raritan Rose peach twigs from inoculation at various dates*

Inoculation date	% nodes showing cankers [a]		Days from inoculation to canker appearance
	Inoculated	Uninoculated	
June 25	60	5	18
July 13	85	4	11
July 24	80	0	9
Sept. 11	80	8	16
Oct. 23	85	0	20
Nov. 20	55	0	90
Dec. 11	9	6	—
Feb. 19	12	9	—
March 19	12	12	—

[a] Based on 200 inoculations at each date.

1) *Leaf scars.*—To assess their importance as infection courts, leaf scars were inoculated with mycelium of *F. amygdali* at intervals from June 25 to March 19. The results (Table 1) indicate a high percentage of scar infection from June 25 to Nov. 20, after which time the scars no longer functioned as infection courts. The period between inoculation and the appearance of cankers lengthened during the cooler, fall months; on the November-inoculated scars it took 3 months or longer for cankers to appear.

During the summer of 1957 2-year-old Early Elberta peach trees were grown in sand culture at 3 levels of nitrogen. In the fall, leaves from these trees were removed 18 days, 11 days, and 1 hour before the trees were sprayed with a Fusicoccum spore suspension. The results (Table 2) show that, at all nitrogen levels, a high percentage of cankers developed when inoculations were made immediately after defoliation. Very few cankers developed when inoculations were made 11 days after defoliation. Since all trees had intact buds at the nodes, and therefore varied only in age of leaf scars, indications are that ingress of the pathogen occurred through the unhealed leaf scars rather than through the buds. This experiment may minimize the importance of buds as infection courts, for the trees were greenhouse-grown and the buds not subject to the bruising that may occur during windy periods in the orchard.

Having confined infection courts to natural and

TABLE 2.—*Canker development on Early Elberta peach trees in relation to nitrogen status and leaf scar age*

N status of tree	Foliage N % of dry weight	Percentage of nodes showing cankers		
		1-hr. scar	11-day scar	18-day scar
High	3.39	51.6	0.0	0.2
Medium	2.53	21.0	0.3	0.8
Low	1.96	26.0	3.5	0.0
		*25.9		

*Significant at the 5% level.

mechanical wounds, a study was undertaken to follow the development of protective substances elaborated at wound surfaces. The abscission of peach leaves normally occurs at a well-defined zone visible to the naked eye. Although the abscissed petiole adjoining the leaf scar contains gum deposits, the newly exposed scar surface is free of gum until about the third day after abscission. Shortly thereafter, suberin begins to accumulate, and within 7–10 days a fully developed periderm is apparent. Periderm development takes longer in early fall than later fall. Appropriate moisture and adequate aeration are necessary for rapid periderm formation. An experiment to determine the effect of humidity on periderm development in peach leaf scars was conducted on trees of varying susceptibility. Twigs of Jerseyland, Triogem, and Sunhigh varieties were placed in chambers maintained at 70–75°F with relative humidity at 50, 60, 70, 80, 90, or 100 per cent. Leaf scars were examined microscopically for gum, suberin, and periderm formation for 16 days. At relative humidities below 90 per cent there was no appreciable difference among varieties, all requiring 4–6 days for gum formation and 7–9 days for full periderm development. At 90 per cent relative humidity there was a 7-day delay in periderm formation in Jerseyland, a particularly susceptible variety. Relative humidity of 100 per cent prevented formation of periderm during the experimental period in all 3 varieties.

Under the conditions of the experiment tabulated in Table 2, gum formation was found within 4 days after abscission, and a fully developed periderm within 7–10 days. Therefore, the virtual absence of cankers in trees inoculated 11 and 18 days after defoliation was attributed to the changes that had occurred in the periderm layer.

2) *Leaves.*—Studies in New Jersey orchards revealed no evidence of twig cankers resulting from leaf infections. Grosclaude (8), however, definitely observed the progress of the fungus through the petiole, with subsequent formation of twig cankers. In New Jersey, twig cankers having been found at the nodes bearing intact leaves on greenhouse trees that had been sprayed with *F. amygdali* spores, inoculations were made on leaves previously punctured with a sterile needle at the tip, base, or elsewhere on the leaf blade. After 31 days cankers appeared at the nodes bearing leaves inoculated at the base. The area of infection could be traced from the puncture, where the fungus gained entrance, through the petiole, and into the twig. Whether, in the orchard, such leaves would persist on the trees long enough to allow twig infection is not certain.

3) *Bud scale scars.*—Leaf and flower buds are protected during dormancy by several layers of overlapping bud scales or modified leaves. As the buds enlarge and the leaves elongate, the scales gradually loosen and are shed, leaving unprotected scars that serve as infection courts in the spring in the same manner as scars left by true leaves in the fall. While the scales are being shed, the old leaf scar tissue also undergoes abscission, but since a new periderm is formed shortly thereafter, opportunity for infection is limited.

TABLE 3.—*Canker development on growing Raritan Rose peach terminal shoots from bud scale inoculations*

Inoculation date	% of terminals with basal cankers [a]	
	Inoculated	Uninoculated
April 20 (petal fall)	38	18
May 1 (shuck split)	18	8
May 8	40	20
June 3	28	16
June 17	30	10
June 22	42	12
July 6	22	10
July 29	0	0

[a] Based on 200 inoculations at each date.

When drops of conidial suspensions were placed in the loosening scale areas at the base of 79 elongating shoots of greenhouse-grown Jerseyland trees, 67 per cent of the shoots developed typical basal cankers. Inoculations were made at the bud scale region of elongating shoots in a Raritan Rose orchard at 2-week intervals beginning at petal fall, in late April, and continuing through late July, when elongation ceased. Data in Table 3 indicate the continued appearance of basal cankers during this period, with a sharp decline at the end of July. Some of the later inoculations required 2–3 months for symptoms to develop. Observation in the orchard of elongating shoots of Raritan Rose and Jerseyland trees corroborated results of inoculation studies, in that basal cankers appeared over an extended season, from June to late August (Table 4). Investigation of the "healing" of bud scale scars revealed a similarity to leaf scars in the development of a protective periderm. When replicated series of Raritan Rose trees covering successive stages of growth were inoculated with a heavy conidial suspension, the largest number of infections developed from inoculations made during the period between the unfurling of leaves and elongation of shoots to 8 inches, this period coinciding with the shedding of bud scales (Table 5).

4) *Buds.*—Wounds resulting from accidental bruising of buds by wind, pruning operations, or other agents also provide a means of ingress for the fungus. Greenhouse experiments were conducted with bouquets of dormant Sunhigh and Raritan Rose branches.

TABLE 4.—*Canker development from natural infection of the growing terminal shoots of 2 peach varieties in the orchard*

Observation date	% of terminals with basal cankers	
	Raritan Rose [a]	Jerseyland [b]
Before June 30	23	5
June 30 to July 28	17	8
July 28 to Aug. 25	5	8
Aug. 25 to Sep. 22	0	0

[a] Based on observation of 350 terminal shoots.
[b] Based on observation of 400 terminal shoots.

gathered from terminals where air turbulence provided opportunity for bruising through collisions with other branches, and from terminals where opportunity for bruising was slight. Whereas the latter were handled very carefully to prevent bruising, the former were handled with less care, allowing further opportunity for bruising before inoculation of the buds with a conidial suspension. Bruised branches of both varieties showed canker development 18 days after inoculation. Neither unbruised bouquet gave evidence of infection by this time. After 25 days, 10.5 per cent of the Sunhigh buds and 5.6 per cent of the Raritan Rose buds from bruised branches showed cankers, whereas 1.2 per cent of the Sunhigh buds and none of the Raritan Rose buds from unbruised branches had cankers.

5) *Fruit scars.*—Orchard investigations have revealed summer infections as well as those occurring in fall and spring. Although there may be some summer infection resulting from late shedding of leaf scales, an important infection court during this period is the fresh fruit scar. Counts made on 200 fruit scars in an unsprayed Raritan Rose orchard indicated that cankers developed at 6 per cent of the fruit scars after both the June drop and the harvest period. Inoculation of fresh fruit scars resulted in cankers at 44–55 per cent of the scars, whereas uninoculated scars showed only 8–12 per cent infection.

Nitrogen status of peach trees.—Since considerable variation in peach canker severity has been experienced in orchards having the same peach varieties on similar spray schedules, differences in nutrition and cultural practices were suspected of influencing incidence of the disease. To evaluate nutritional effects, 2-year-old Early Elberta trees were grown in sand culture on varied nitrogen treatments. A basic nutrient solution—containing 15.5 ppm phosphorus, 70 ppm potassium, 46 ppm magnesium, 160 ppm calcium, and adequate amounts of the minor elements—was modified to provide 3 nitrogen levels: 14 ppm, 56 ppm, and 112 ppm. In the fall, when the trees were reflecting their respective treatments, replicate trees of each group were defoliated 18 days, 11 days, and immediately before being sprayed with a conidial suspension of *F. amygdali.* After 13 days cankers started to appear at the nodes of all trees except those low in nitrogen, which did not show recognizable cankers until the 29th day after inoculation. As early as 16 days after the cankers became visible on these trees, pycnidia and oozing tendrils were observed; not until much later were tendrils observed on the medium- and high-nitrogen trees. Low nitrogen being related to high carbohydrate in plant growth, the high sugar content was considered responsible for early fruiting in these trees. Data in Table 2 indicate a significantly greater percentage of cankered nodes in the high-nitrogen trees than in trees of other treatments.

Histological studies of the leaf scars were made daily after defoliation of each nitrogen group. The leaf scars were found to vary only slightly in the time required for gum and periderm development. All showed gum development by the fourth day after defoliation, and all showed a complete periderm after 7 days, except those on low nitrogen, which required 10 days for full periderm development.

TABLE 5.—*Incidence of infection from inoculations of trees at various stages of development*

Tree stage at inoculation	% inoculated nodes developing bud or basal cankers [a]	% inoculated shoots developing basal cankers [a]
Delayed dormant	6.8	2.4
Vegetative buds just showing green	8.0	3.5
Buds ½-in. green	12.0	17.2
Leaves unfurling	22.6	31.0
Buds 1–3 in. of green	25.3	39.6
Shoots starting to elongate	25.0	43.0
Shoots 4–6 in. long	47.8	50.0
Shoots 6–8 in. long	27.5	41.0
Shoots 8–10 in. long	6.7	10.0
Shoots 8–12 in. long, maturing	1.9	4.4

[a] Based on inoculation of 4 2-year-old trees per treatment.

Control studies.—1) *Laboratory tests.*—Preliminary to orchard tests for canker control, laboratory studies were conducted to determine the effectiveness of fungicides commonly used for control of peach canker (5, 8, 11). Each fungicide was added in varying concentrations to plates of agar media inoculated with Fusicoccum mycelium and incubated at 80°F. At a concentration of 0.0001 per cent, captan, ferbam, dichlone, thiram, ziram, and maneb either entirely prevented or allowed only slight growth of the fungus. Sulfur was non-toxic to mycelial growth of *F. amygdali* at a concentration of 1 per cent, and the coppers and Glyodin were intermediate in toxicity.

Studies were also conducted to determine the effect of these fungicides on spore germination. Serial dilutions were made of each commercial preparation. Captan, thiram, Dyrene, and Omadine were extremely toxic, permitting no germination of *F. amygdali* spores at a concentration of 0.0000001 per cent. Dichlone, lead arsenate, ziram, ferbam, and Agricultural Puratized were effective at 0.00001 per cent. Bordeaux, wettable sulfur, maneb, zineb, Glyodin, mono-calcium arsenite, calcium cyanamid, and tank-mixed zineb showed relatively little control, and Copper "26" was completely ineffective against the organism. As a group the carbamates showed greater activity than the ethylenebisdithiocarbamates.

This same group of fungicides was applied to freshly defoliated greenhouse trees whose leaf scars were examined every 2 days for gum and periderm development. Captan and Bordeaux were found to accelerate normal periderm development, whereas lime sulfur delayed its development. Other chemicals, including ferbam, tank-mixed zineb, dichlone, calcium cyanamid, Omadine, sulfur, maneb, thiram, and Dyrene, had no effect on periderm development. In light of these results, materials showing good fungicidal activity

without adversely affecting periderm development would be most acceptable in a control program.

2) *Orchard trials.*—Although an effective fungicide is important throughout the growing season, emphasis should be given to control measures during the spring, early summer, and fall peaks of infection (5, 6, 8). In the fall of 1952–53, control experiments were conducted on 2 blocks of 8-year-old Jerseyland peach trees. Block A was sprayed with a speed sprayer, and Block B by hand. Since leaf drop in New Jersey extends from August through November, the sprays were applied at 2-week intervals during this period. Materials effective in the laboratory, i.e. carbamates, dichlone, and captan, were used in addition to sulfur, which is a common peach fungicide. Canker counts were made on 100 sucker terminals taken from the center of 20 trees on December 16, March 17, and at shuck-off (Table 6). On December 16, cankers were found on all trees, many having been present on current-season twigs at the start of the test. Although there were no significant differences among the fungicide treatments, all had fewer cankers than the unsprayed trees. Counts made on March 17 showed a considerable increase in the incidence of cankers after December, except in the tank-mixed zineb treatment, which had caused early defoliation. Throughout the spring all trees were sprayed at weekly intervals with ferbam (2 lb./100 gal.) beginning at the early pink stage and continuing through the blooming period. Counts made at shuck-off revealed an increase of 12–72 cankers per 100 terminals after the March count. The prematurely defoliated trees again were among those developing the fewest cankers. Since the entire block was on a uniform treatment during the spring, differences between treatments might be attributed to the fact that some of the lesions that appeared in the spring were actually the result of fall infections (8). That fall infections may not make their appearance until the following spring is further illustrated by the fact that bouquets gathered in the orchard during the late dormant period developed additional cankers when they were held in warm temperatures in the greenhouse.

In Block B, canker counts were made in December and at shuck-off. Here again, the increase in number of cankers after the December count varied considerably between treatments (Table 7). Again the defoliated trees showed the smallest increase in cankers. Since all trees in both blocks had been pruned of dead and diseased wood in early spring, these differences are not believed to be due to variation in spore loads in the various trees.

With the hope that foliage removal in early fall might reduce the number of applications needed for canker control, attempts were made to find a defoliant that would remove the foliage in a short time without injuring the bark or retarding periderm development. In this quest, several defoliants were tested, including endothal (disodium salt of 3,6-endoxohexahydrophthalic acid), lime sulfur, liquid dithane (nabam), copper sulfate solution, zinc sulfate solution, calcium cyanamid dust, and liquid hydrogen cyanamid applied alone and after a lime spray. These chemicals removed most of the foliage; severe injury resulted, however, from the use of endothal and copper and zinc sulfates. Only the cyanamids proved to defoliate efficiently without causing bark injury. Microscopic studies showed that they did not interfere with periderm development. Calcium cyanamid dust (40 lb./acre) removed about 95 per cent of the foliage in 6 days, whereas liquid hydrogen cyanamid (2 gal./100 gal.) following a lime spray gave complete defoliation in 4 days.

Further orchard experiments were conducted, with the following fungicides: ferbam, thiram, maneb, zineb, and captan, all at 2 lb. per 100 gal.; dichlone (1 lb./100 gal.); wettable sulfur (5 lb./100 gal.); tank-mixed zineb (2 qt. nabam + 1 lb. $ZnSO_4$); and Bordeaux mixture (2-6-100). Each fungicide was applied to 20 randomized trees at 10–14-day intervals from mid-August until mid-October, at which time 10 trees from each treatment were sprayed with hydrogen cyanimid (2 gal./100 gal.). Application of the defoliant to trees sprayed with Bordeaux mixture resulted in complete defoliation within 4 days; whereas, following any of the fungicides not containing lime, it resulted in about 95 per cent defoliation within 4–6 days, 5 per cent of the foliage remaining until frost. A

TABLE 6.—*Canker development in an orchard of Jerseyland peaches (block A) given various fungicide treatments*[a]

Material (lb./100 gal.)	No. of cankers/100 terminals[b]				
	Dec. 16	March 17	Shuck-off	Dec.–shuck-off	March–shuck-off
Check, unsprayed	74	115	174	100	59
Sulfur, 5	41	61	113	92	52
Ziram (76%), 2	32	48	64	32	16
Ferbam (76%), 2	33	43	61	28	18
Thiram (65%), 2	47	63	75	28	12
Captan (50%), 3	31	39	51	20	12
Zineb (65%), 2	31	57	73	42	16
Dichlone (50%), 1	35	73	90	55	17
Nabam (19%) + $FeSO_4$ + lime, 2 qt.–10 oz.–1 lb.	29	45	73	44	28
Nabam (19%) + $ZnSO_4$ + lime, 2 qt.–1 lb.–1 lb.	37	39	53	16	14

[a] Blocks were sprayed with Ferbam (2 lb./100 gal.) from pre-bloom to shuck-off, followed by specific fungicides on a 2-week schedule from August 13 until completion of leaf drop.

[b] Based on 20 trees per treatment.

TABLE 7.—*Canker development in an orchard of Jerseyland peaches (block B) given various fungicide treatments* [a]

Material (lb./100 gal.)	No. of cankers/100 terminals [b]		
	Dec. 9	Shuck-off	Dec. 9–shuck-off
Check, unsprayed	141	293	152
Sulfur, 5	125	249	124
Ziram (76%), 2	52	96	44
Ferbam (76%), 2	79	107	28
Thiram (65%), 2	108	140	32
Captan (50%), 3	39	73	34
Zineb (65%), 2	59	111	52
Dichlone (50%), 1	69	107	38
Nabam (19%) + $FeSO_4$ + lime, 2 qt.–10 oz.–1 lb.	51	99	48
Nabam (19%) + $ZnSO_4$ + lime, 2 qt.–1 lb.–1 lb.	40	61	21

[a] Blocks were sprayed with Ferbam (2 lb./100 gal.) from pre-bloom to shuck-off, followed by specific fungicides on a 2-week schedule from August 13 until completion of leaf drop.

[b] Based on 20 trees per treatment.

dry period from the time of forced defoliation until periderm formation in the scars precluded the need for further fungicide applications on these trees.

The trees were examined for cankers the following spring at the shuck-split stage. Although there were no significant differences among the fungicide treatments, their average number of cankers per 10 ft. of twig growth was 2.8, compared with 7.7 cankers per 10 ft. for the unsprayed trees. A significant improvement was also exhibited by forced defoliation, wherein the use of hydrogen cyanamid resulted in 3.6 cankers for every 10 ft. of twig growth, as against 5.9 cankers per 10 ft. for the non-defoliated trees. Forced defoliation is a technique that may prove of value in the control of any disease where fresh leaf scars serve as infection courts.

In an attempt to control spring and early summer infection, 7 rows of Raritan Rose trees were sprayed during the advanced delayed dormant stage with a mixture of monocalcium arsenite (3 lb.), Bordeaux (4-6-100) and oil (1½ pt.) (per 100 gal. of spray). By mid-summer, flagging limbs began to appear as a result of girdling by basal cankers. Flagging continued through the entire fall season, and many of the infected limbs died during the ensuing months. Counts made in September revealed an average of 56.4 girdled twigs per tree in the unsprayed portions of the block, and 2.4 in sprayed portions. Arsenical injury to the 2-year wood was common on the rows sprayed with monocalcium arsenite.

Sprays applied during the early-pink, bloom, and early-cover periods rarely caused a significant reduction in bud cankers. Perhaps this was due to the fact that many cankers developing in the spring of the year are the result of fall infections. Spring sprays, however, occasionally showed control.

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WINTER TREATMENTS OF DORMANT ROSES AND THEIR RELATION TO BLACKSPOT AND SPIDER MITE CONTROL [1]

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SUMMARY

Roses in 120 plots of 5 varieties each were used to determine the effect of dormant-cane spraying, ground spraying, pruning, and clean culture (weed and rose leaf removal and a sawdust mulch) on the severity of blackspot, caused by *Diplocarpon rosae* Wolf, and of two-spotted spider mites (*Tetranychus telarius* (L.)). Pruning canes to within 1–2 in. of the bud union was the most effective. A dormant-cane spray application of lime-sulfur of any of 3 organic mercury fungicides, of an antibiotic (Actidione), or of monocalcium meta-arsenite was ineffective. Ground spraying with Elgetol and clean culture also were ineffective. Spider mite populations were reduced by clean culture, which destroyed their over-wintering weed hosts. Severely pruned rose plants produced few flowers early in the season, but as many as the unpruned bushes by the end of the season.

Various winter treatments as well as summer practices have been suggested for control of blackspot, caused by *Diplocarpon rosae* Wolf, and of two-spotted spider mites (*Tetranychus telarius* (L.)) on outdoor roses. Aronescu (2), Frick (5), and Wolf (17) concluded that, in cold climates, the blackspot fungus overwinters on fallen leaves and that spring infection takes place largely from spores produced on infected dead leaves lying on the ground. Alcock (1) was the first to suggest that blackspot on the canes would serve as a source of spring infection. Ewing (4) showed that spider mites overwinter on green foliage of weeds and other plants and that clean culture prevents heavy spring infestations in hops and other crop plants. These and other reports prompted such suggested treatments as clean culture (removing old leaves from rose plants and beds and removing weeds) and application of sprays to the dormant canes and to the soil (3, 4, 5, 9, 12, 13, 15 p. 88–93, 17).

Because of the divergent opinions in the references cited, and because few experiments had been designed to determine the efficacy of various treatments, tests of their effectiveness were made in the winter of 1955–56 at Beltsville, Maryland.

MATERIALS AND METHODS.—Used in these tests were 120 rose plots on which insecticide-miticide-fungicide dust combinations had been compared in 1954 and 1955 (10, 14). Each plot consisted of 16 plants, including 5 varieties of roses. After frost in the fall of 1955, a muslin barrier 3 ft. high was erected around each plot, with walkways between replicates to facilitate treatment. The plots were divided into 3 groups:

1) 48 plots of plants weakened by moderate to severe blackspot infection in 1954 and 1955. During these years the plants in these plots had received no fungicide or received 3.4 per cent copper (from basic copper sulfate) or 1 per cent Karathane (2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and isomers).

2) 48 plots of vigorous plants that had received good blackspot control in 1954 and 1955: dusted with copper-sulfur (3.4 per cent copper from basic copper sulfate and 25 per cent sulfur); ferbam-sulfur (7.6 per cent ferric dimethyldithiocarbamate and 25 per cent sulfur); 6 per cent zineb (zinc ethylenebis[dithiocarbamate]); or 7.6 per cent ferbam dust.

3) 24 plots of plants of intermediate vigor that had received fair blackspot control in 1954 and 1955: dusted with 5 per cent captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) or 25 per cent sulfur.

Plots in groups 1 and 2 were used for a factorial experiment in which some of the higher order interactions were confounded. Treatment combinations were as follows:

(a) Cane sprays. Dormant rose canes were sprayed with a 12-gallon Myers Silver Prince power sprayer at 125-lb. pressure until the spray ran off. Some of the spray material drifted from the plants onto the ground or onto the sawdust beneath the plants. An average of ½ gal. of spray material was used for each 144-sq.-ft. plot of 16 plants. Two spray materials were used: A_1, 1:8 lime-sulfur and water; and A_2, ½ lb. Coromerc (10 per cent *N*-phenylmercuriethylenediamine) per 100 gal. of spray solution. The concentration of lime-sulfur is commonly recommended for use on roses (15), and Coromerc has shown promise as a dormant spray for control of scab of almonds (16). A_1 was applied February 9, 1956, and A_2 on February 13.

(b) Ground spray. The soil surface or sawdust and fallen leaves were sprayed with 0.5 per cent Elgetol (32 per cent sodium 4,6-dinitro-*o*-cresoxide) on February 13, 1956. The force of the spray stream tended to blow the leaves around, and the spray may have missed a few. In general, however, coverage was good. About 1⅓ gallons of spray was applied per plot.

(c) Heavy pruning. Plants in some plots were severely pruned to 1–2 inches above the bud union on February 9, 1956. All prunings were removed from these plots. Plants in other plots were not pruned until early summer, when all dead wood was pruned out and removed.

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TABLE 1.—*Blackspot ratings on Golden Masterpiece roses (June 13, 1956) and spider mite counts (May 28-29, 1956) on Red Radiance rose leaves following winter treatments of rose plots*

Treatment	Blackspot rating [a]	No. of spider mites [b]
Cane sprays:		
No fungicide	15.0	299
Lime-sulfur	10.2	410
Coromerc	13.9	415
Ground sprays:		
None	12.7	404
Elgetol	13.3	346
Pruning:		
None	25.0	326
Severe	1.1	424
LSD (1% level) [c]	4.7	123 [d]
Culture:		
None	12.9	449
Clean	13.2	301
LSD (1% level) [c]		123

[a] Average percentage of leaves showing blackspot per plot.
[b] Total number of mites on 5 $\frac{9}{16}$-in. leaf disks per plot.
[c] An LSD is given only where differences between treatments were statistically significant.
[d] Differences are significant at the 5% level.

(d) Clean culture. Old foliage was raked up, weeds hoed out, and fresh sawdust applied over the entire plot to a depth of 1–2 inches on February 9, 1956. These plots were kept free of weeds and trash until June.

Group-3 plots were used in a 6-replicate experiment comparing 4 fungicides as rose cane sprays: 1) Panodrench (0.6 per cent cyano(methylmercuri) guanidine), 187 ml per 100 gal.; 2) Phix (22 per cent phenylmercury acetate), 0.25 lb. per 100 gal.; 3) Acti-dione (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide), 2.5 ppm; and 4) 80 per cent mono-calcium meta-arsenite, 3 lb. per 100 gal. Each spray mixture contained 187 ml of Santomerse per 100 gal. On March 4, 1956, the rose canes were thoroughly sprayed until the spray ran off. The temperature was 60–65°F and the sun was bright.

All barriers were left in place until the middle of June. On June 13 the percentage of leaves showing blackspot on the Golden Masterpiece variety was independently estimated by 3 observers. Blackspot data were obtained only for Golden Masterpiece because differences in amount of blackspot were greatest in this variety. Spider mite populations were sampled on May 28–29, 1956, by cutting 5 $\frac{9}{16}$-in.-diameter leaf disks from fully expanded leaves of the Red Radiance plants in each plot and counting the numbers of spiders on these disks.

After blackspot records were obtained in June, all plots were sprayed each week during the rest of the growing season with a zineb-DDT-lindane combination. DDT is commercially available dichlorodiphenyltrichloroethane, the principal constituent of which is 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane; and lindane is γ-1,2,3,4,5,6-hexachlorocyclohexane. Systox (containing 25 per cent of a mixture of 0,0-diethyl-S-ethylmercaptoethyl thiophosphate and 0,0-diethyl-O-ethylmercaptoethyl thiophosphate) was added every 2 weeks for mite control.

Flowers were cut twice each week during the growing season and the numbers recorded by plot and variety. Records were made for Golden Masterpiece and Red Radiance. The other 3 varieties, Chief Seattle, Peace, and Christopher Stone, were pulled from the plots in June.

RESULTS.—The blackspot ratings on Golden Masterpiece and the spider mite counts on Red Radiance in the factorial experiment (Table 1) show no effect of the dormant-cane sprays or of the ground sprays. Cane sprays gave a slight, but not significant, reduction in blackspot severity, and a slight, but not significant, increase in mite populations. The annual weeds, particularly *Erigeron canadensis* L. and *Stellaria media* (L.) Cyrill., served as overwintering hosts for the mites (14). Keeping the plots free of weeds, removing old leaves, and adding a fresh sawdust mulch reduced mite populations but not blackspot. Such results confirm the work of Ewing (4). On the other hand, pruning the roses resulted in increased mite populations, probably because the reduced amount of foliage resulted in concentration of the mites on the remaining foliage. Pruning was the only dormant treatment that reduced blackspot significantly. It is well known that *D. rosae* commonly overwinters on rose canes (6).

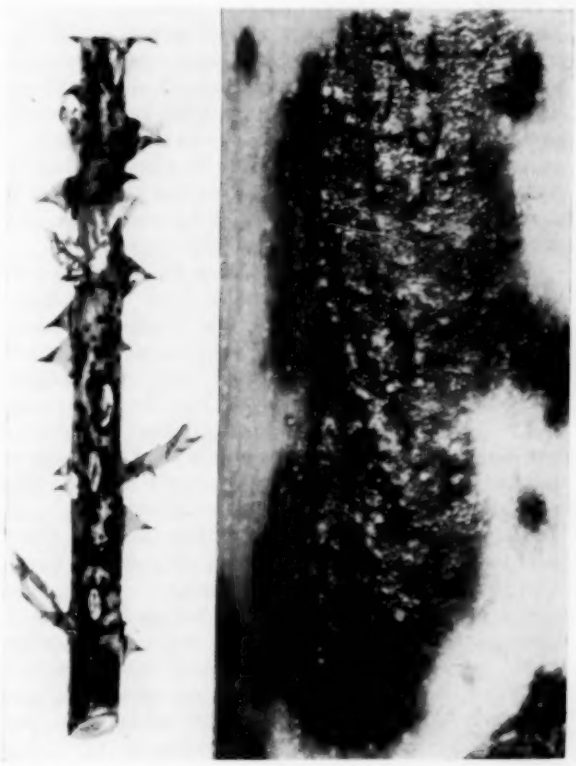

FIG. 1. Blackspot lesions on Show Girl rose canes, April 23, 1956. Left, cane with numerous lesions; right, closeup of a single lesion, showing the acervuli (×10).

TABLE 2.—*Yield of flowers in 1956 by heavily pruned (February 9, 1956) and unpruned roses that had been either moderately or severely affected by blackspot in 1954 and 1955*

Variety and blackspot history	Treatment	Av. no. flowers per plot	
		June 1–July 13	July 16–Sep. 28
Red Radiance			
Moderate	Unpruned	174	177
	Pruned	82	211
Severe	Unpruned	98	131
	Pruned	57	124
Golden Masterpiece			
Moderate	Unpruned	83	58
	Pruned	31	50
Severe	Unpruned	18	24
	Pruned	12	29

Lesions such as those shown in Fig. 1 were numerous on the canes of the Golden Masterpiece and the Red Radiance roses in this experiment. The extent to which these overwintering cane lesions serve as a source of infection in the spring, however, had not been measured previously. Pruning roses nearly to the ground (within 1–2 in. of the bud union) nearly eliminated early blackspot infection, whereas it was very severe on unpruned roses.

Since all 4 sprays applied to dormant canes in a separate experiment were ineffective in controlling blackspot, the data from these plots are not presented.

Flower yield records (Table 2) were obtained to determine whether severely pruned plants would develop with relatively little blackspot and eventually outproduce unpruned, severely diseased plants. Plants with severe blackspot in one year have been shown to produce fewer flowers at the beginning of the next season than do moderately diseased plants (11). In these tests, unpruned bushes of both varieties produced more flowers early in the season, but no more than the pruned bushes during the last of the season.

DISCUSSION.—The data presented do not show any control of blackspot by clean culture or dormant sprays, but they do raise a number of questions about the value of pruning. We showed previously (11), and Lyle (8) reported, that control in one year delayed and decreased the incidence of blackspot the next. Lyle (7) also delayed the onset of blackspot during rose propagation by delaying removal of the tops of resistant understocks until early April and pruning back all prematurely formed scions to less than ½ in. He stated that this delay lessened the amount of fungicidal treatment needed. Results of the current experiment indicate that, under conditions at Beltsville, cane lesions are primarily responsible for early blackspot development; this finding suggests that more attention should be given to reducing the number of cane lesions. Our experiments showed that severe pruning will reduce early blackspot, but not whether moderate or normal pruning will be effective, as Massey (13) suggested. These experiments indicate that blackspot can be reduced to a low level and the plants maintained relatively disease-free by severe pruning followed by a good fungicidal spray or dust program. Further experiments are needed to determine the effect of late-fall sprays on cane infection and on subsequent blackspot development, the effect of normal pruning on blackspot development, the effect of a spray program begun soon after pruning, and whether the addition of wetting agents to cane sprays to facilitate penetration of the cane lesions by the fungicides would be beneficial.

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FACTORS AFFECTING THE VIRUS CONTENT OF CHEYENNE WHEAT INFECTED BY WHEAT STREAK MOSAIC [1]

Ernst Haunold [2]

SUMMARY

The concentration of wheat streak mosaic virus in extracts of diseased wheat seedlings, grown under different environmental conditions, was determined by infectivity assays. The time required for the virus to reach maximum concentration decreased with an increase in temperature, being 10 days for 24°C or above, and 20 days for 20°C; at 12°C the virus concentration remained low for 30 days. Day length and light intensity had no perceptible effect on virus content. Concentrations of 630 and 1050 ppm of nitrogen in nutrient yielded more virus 10 days after inoculation than did 70 ppm; phosphorus levels of 547 ppm produced more virus than did 237 and 10 ppm. Differences in virus concentration decreased at longer times. Potassium did not perceptibly influence virus content.

The importance of environment on the virus content of infected plants is becoming increasingly evident. Much literature exists on virus multiplication in several rather important economic plants, especially tobacco, potato, and some vegetables, but nothing has been reported on grasses. Wheat streak mosaic virus was reported to occur in relatively low concentrations in host plant juice (4) and to show a great deal of instability when purified (2). For that reason, virus assays in the present work are based on infectivity data of the expressed plant juice. This is a study of the effect of environment on virus concentration in plants infected with wheat streak mosaic.

MATERIALS AND METHODS.—The host plants used in this work were young Cheyenne winter wheat seedlings in the third-leaf stage. The plants were grown in composted soil in 4-in. pots in a greenhouse at 24 ± 2.5°C. The inoculum was a yellow-mottling strain of wheat streak mosaic virus.

Temperature studies were carried out at 12, 20, 24, 28, 30, and 32°C in thermostatically controlled chambers in the greenhouse. Temperature variation was ordinarily ±1.5, but sometimes as high as ±4°C. There were 18 pots of 10 plants each in each chamber. One half of the seedlings were inoculated with the virus, and the other half with tap water only. Plants were placed in the chambers the day after inoculation. These experiments were carried out in January–July and September–October, 1956.

Light-intensity and day-length studies were carried out in controlled chambers where the temperature was regulated at 24 ± 1°C. Two chambers received high light intensity (2000 foot candles) and day lengths of 24 and 16 hours, respectively. In 2 other chambers the light intensity was 620 foot candles, with the same day lengths. The light was supplied by fluorescent light tubes (30 W). The number of plants in each chamber and the treatment of seedlings until placed in the chambers were as in the temperature studies. The experiments were conducted in June–July, 1956, and March–April, 1957.

The effect of host nutrition on virus accumulation was studied from September, 1956, to May, 1957. The wheat was grown in sand that had been washed with acid and water and placed in 5-in. pots coated inside with an asphalt paint. On emergence, the seedlings were supplied a balanced nutrient solution (1H) formulated by Hoagland and Snyder (7). Each pot received 100 ml of nutrient solution every other day. When necessary, distilled water was added between feedings. The nutrient solutions were applied when the plants had reached the third-leaf stage. For 3 days before the change in nutrient solution, the plants were flooded periodically with distilled water to wash out any traces of the previous nutrient solution. The plants were inoculated 1 day after the specific solutions were applied. The composition of the specific solutions, containing different amounts of N, P, and K, was taken from Cheo, Pound, and Weathers (3). The solutions were adjusted to pH 6.0, except for the phosphorus solutions (kept at pH 5.5). There were 12 pots of 20 plants each for each solution studied.

All plants were inoculated by leaf rubbing with a cheesecloth pad in the third-leaf stage after dusting with 600-mesh carborundum. The inoculum was sap expressed from 10 g of infected tissue to which had been added 10 ml of buffer, pH 6.2, prepared from 0.2M disodiumphosphate and 0.1M citric acid.

One third of the plants were cut at the soil level and weighed 10, 20, and 30 days after inoculation. The diseased plants were assayed for virus infectivity. Ten g of tissue, cut into small pieces with scissors, was selected randomly for inoculum, prepared as described above. After the original dilutions with buffer, the virus preparations were diluted with distilled water and inoculated in the following concentrations: undiluted, 1:10, 1:100, 1:1000, and 1:10,000. The percentage of infected plants was recorded after 15 days. The infectivity data were evaluated according to the method of Plus (9) and Brakke (1). The loglog $\frac{100}{\%\text{ infected plants}}$ was plotted against the logarithm

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TABLE 1.—*Formula for the specific nutrient solutions.*

Salt	Molarity stock solution	Stock solution (cc) per liter of final solution to get concentration (ppm) of								
		Nitrogen			Phosphorus			Potassium		
		70	630	1050	10	237	547	78	430	704
$Ca(NO_3)\cdot 4H_2O$	2.5		4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
KNO_3	2.0	2.50	2.50	2.50	2.50	2.50	2.50		2.50	2.50
$NaNO_3$	6.0		5.00					0.83		
NH_4NO_3	6.0			5.00						
KCl	3.0				0.22			0.67	2.00	4.00
KH_2PO_4	1.0	1.00	1.00	1.00	0.33	1.00	1.00			1.00
$MgSO_4\cdot 7H_2O$	2.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$CaCl_2\cdot 2H_2O$	4.0	1.25								
$NaH_2PO_4\cdot H_2O$	2.0					3.33	8.33	0.50	0.50	
NaCl	5.0	8.50	2.00	2.00	6.09	4.00	1.00	5.59	4.79	3.59

of dilution, and the best straight line with slope −1 was drawn through the points. The distance between two lines gives the logarithm of the ratio of virus concentration in 2 extracts. For comparison, all inocula were referred to that dilution where 63 per cent of the assay plants would become infected. Comparison of these values with those calculated by the maximum likelihood method for sample assays, indicated that the present values would very seldom have a maximum error greater than ±0.3 log unit (1). Most of the present assays appeared to give a satisfactory fit to a straight line with slope −1 when plotted in the above manner. There is possible additional error of unknown value in comparing assays made at different times.

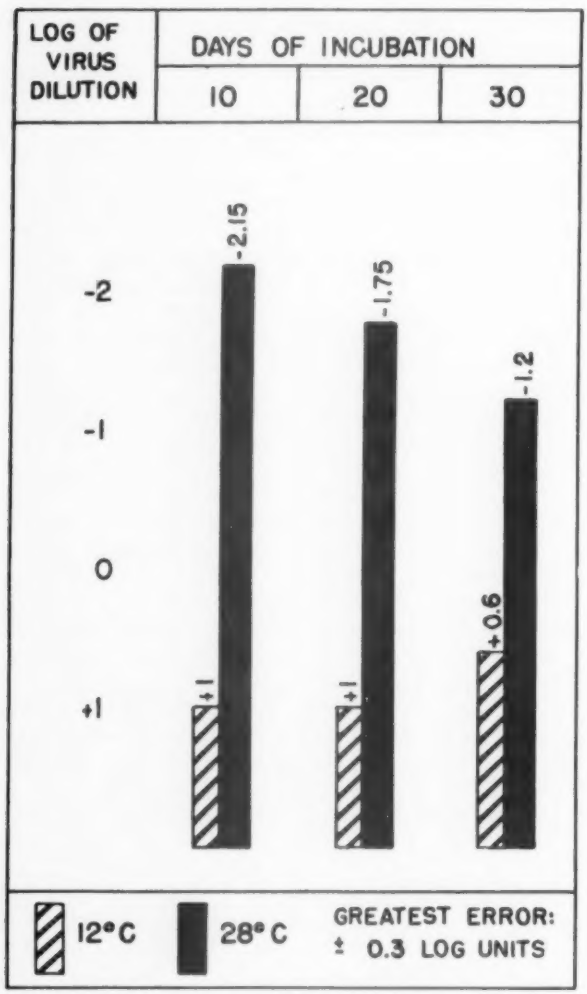

FIG. 1. Effect of low and high temperature on virus concentration of wheat streak mosaic virus in plants. Dilution of virus preparations that would give infections of 63% of the inoculated plants.

RESULTS.—*Effect of temperature on virus content and plant growth.*—At 10, 20, and 30 days after inoculation, respectively, virus concentration in extracts of plants grown at 28°C was 3.15 ± 0.6 log units, 2.75 ± 0.6 log units, and 1.8 ± 0.6 log units higher than that in plants grown at 12°C (Fig. 1). Plant growth was less at 12°C than at 28°C; infected plants grew less than the respective checks. Subsequent experiments at 20, 24, 28, 30, and 32°C showed that the virus multiplied rapidly at 20°C and above. At 24, 28, and 30°C the virus reached its peak after the first 10 days. At 28°C and above, a decrease in virus level was observed toward the end of the experiment. At 32°C, many of the test plants died because of excessive heat, but the survivors had a fairly high virus content at 10 days, when the experiment was discontinued. Since these last experiments were not run simultaneously, the accuracy of the comparisons is uncertain.

Another series of experiments was conducted simultaneously—at 20, 24, and 28°C (Fig. 2, A), which appeared from the above results to be the most promising range. Assay for virus content was restricted to the 2 or 3 youngest leaves of the seedlings. At the end of the experiment (30 days), the young leaves and the remaining plant parts were separately assayed. Similar virus concentrations were reached in all the young leaves of the plants, irrespective of temperature. Maximum virus concentrations were attained after the first 10 days, and remained the same until the experiment was terminated. Thirty days after inoculation, the stems and old leaves of plants at 20°C contained 0.9 ± 0.6 log units more virus than those at 28°C; virus content at 24°C was intermediate (Fig. 2, A). Plant growth was best at 20°C, and decreased

with increasing temperature. Infected plants made less growth than the respective checks.

Effect of photoperiod and light intensity on virus concentration and plant growth.—Neither day length nor light intensity significantly influenced the virus content of the plants. Maximum virus concentration was reached at 10 days, and remained at that level throughout the course of the experiments. Plant growth was greatest at greatest light intensity and longest photoperiod.

Effect of host nutrition on virus content and growth of the plants.—1) *Nitrogen.*—The concentrations of nitrogen used in each of 2 experiments were 70, 630, and 1050 ppm. In experiment 1, plants at the low nitrogen level contained 0.8 ± 0.6 log units less virus 10 days after inoculation than those at the 2 higher nitrogen levels (Fig. 2, B). The difference became 1.1 ± 0.6 log units by 20 days. At the end of the experiment (30 days) a difference (0.6 ± 0.6 log units) in virus content existed only between plants at the lowest and highest nitrogen levels. In experiment 2, the measured differences were less pronounced (Fig. 2, B). The discrepancy may partly reflect an inaccuracy of assay. Plant growth was correlated with the amount of nitrogen supplied, and the infected plants grew less than the checks.

2) *Phosphorus.*—Phosphorus solutions were used at 10, 237, and 547 ppm in 2 experiments. Plants at 547 ppm contained 0.73 ± 0.6 log units (average of 2 experiments) more virus than those at 10 or 237 ppm. Assays at 10, 20, and 30 days showed little difference. The growth of healthy plants was only slightly influenced by amount of phosphorus. In both experiments, infected plants grew best in the order 237, 10, and 547 ppm.

3) *Potassium.*—The 3 levels of potassium used in the 2 experiments were 78, 430, and 704 ppm. Amount and time had little influence on virus content or growth. Infected plants showed less growth than the checks.

Discussion.—The data suggest that no specific temperature is necessary for wheat streak mosaic virus to reach maximum concentration in the host, but temperature will affect the time required. According to Sill (11), symptoms of wheat streak mosaic virus developed in 5, 7, 9, and 15 days at temperatures of 28, 24, 20, and 16°C, respectively. Severest symptoms appeared first at 28°, but shifted to 24°, then to 20°, and in 2 months to 16°C. In the present experiments the lowest virus concentration existed in the plants at 12°C. The rapid attainment of maximum virus content at higher temperatures may have resulted from a greater multiplication rate of virus at the higher temperatures and, possibly, a better translocation of the virus throughout the host, resulting in infection of an increased number of cells, which in turn would produce more virus. The lower virus content toward the end of the experiment at the higher temperatures of 28°C and above was probably an aging effect. Plants age faster at higher than at lower temperatures; more fibrous tissues are present, which makes virus extraction more difficult. The metabolic changes in an aging plant cell may induce a degradation of the virus. In addition, high temperatures may cause a breakdown of the proteins, as described by Harrison (5) and Kassanis (8).

High concentrations of nitrogen (630 and 1050 ppm) and phosphorus (547 ppm) gave the greatest virus yields at 10 days after inoculation. It was assumed that virus synthesis occurs most rapidly in plant cells

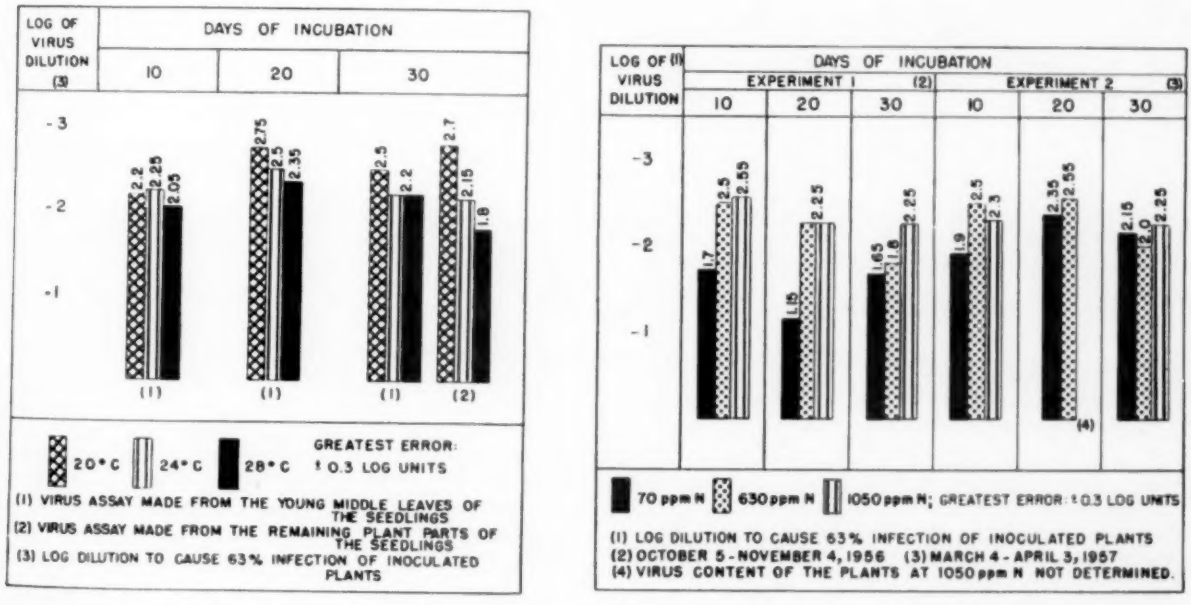

Fig. 2. Virus infectivity assays of wheat-streak-mosaic-infected Cheyenne wheat. A) At different temperatures. B) At different nitrogen levels.

that have a high metabolic activity as a result of luxurious food supply. Both phosphorus and nitrogen are essential parts of the virus complex, and their ready availability may be of great significance for virus synthesis. The lower virus concentration 10 days after inoculation in plants receiving less nitrogen or phosphorus may have been due to lower availability of metabolic products. A gradual increase in virus led to almost the same concentrations at 20 and 30 days after inoculation as in plants that received ample amounts of nutrients. The nutrient concentrations supplied to the host were not so low as to stop plant growth.

Virus production in relation to nitrogen supply has been reported either to be correlated with host growth (3, 10) or to increase with amounts of nitrogen supplied even when the plants became stunted (6, 12). In the present studies the plants grew best at 630 and 1050 ppm, when virus yields were also greatest. And at the highest phosphorus level also, virus yields were greatest. The results are in general agreement with those of other workers (3, 10), who reported highest virus yields at highest phosphorus levels even though plant growth was decreased. The findings of the present investigations differ in 1 respect: highest virus yields were regularly obtained only after the first 10 days. A significant importance of potassium on virus yield has not been reported so far (3, 10, 12).

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ADDITIONAL STUDIES ON THE INHERITANCE OF RESISTANCE TO FROG-EYE LEAF SPOT OF SOYBEANS [1]

A. H. Probst and Kirk L. Athow

SUMMARY

Resistance to frog-eye leafspot in the soybean varieties Lincoln, Illini, Wabash, and experimental strain C1076 was found to be controlled by the same gene.

There was no evidence that modifying factors act on resistant genes to condition the inheritance of the intermediate reaction of the varieties Perry and Chief. It could not be determined from these studies whether there was an intermediate gene or a modification of the gene for susceptibility.

Resistance of soybean to frog-eye leaf spot incited by *Cercospora sojina* Hara was reported by Athow and Probst [2] to be due to a single dominant Mendelian factor. They found Lincoln and Wabash varieties resistant; Gibson, Patoka, and Hawkeye highly susceptible; and Chief and Perry somewhat less susceptible (intermediate). The difference between the highly susceptible and intermediate varieties was in number of lesions per plant rather than size of lesion or abun-

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Contribution from the Department of Agronomy and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana, and U. S. Regional Soybean Laboratory, Crop Research Division, Agricultural Research Service, U. S. Department of Agriculture, Journal Paper No. 1228, Purdue University Agricultural Experiment Station. Journal Paper No. 312, U. S. Regional Soybean Laboratory.

[2] Athow, Kirk, and A. H. Probst. 1952. The inheritance of resistance to frog-eye leaf spot of soybeans. Phytopathology 42: 660-662.

TABLE 1.—*Segregation for reaction to Cercospora sojina in the F_2 population of 18 soybean crosses*

Reaction class and parentage	Number of F_2 plants			3:1 ratio	
	Total	Resistant	Susceptible	χ^2	P
Resistant × Resistant					
Illini × C1076	158	158	0		
Wabash × Lincoln	217	217	0		
Wabash × Illini	196	196	0		
Wabash × C1076	184	184	0		
Total	755	755	0		
Resistant × Susceptible					
Gibson × C1079	198	139	59	2.43	.20–.10
Gibson × Illini	196	147	49	0.00	.99
Gibson × Wabash	169	131	38	0.57	.50–.30
Hawkeye × C1076	140	100	40	0.95	.50–.30
Illini × Hawkeye	201	149	52	0.08	.80–.70
Wabash × Hawkeye	222	164	58	0.15	.70–.50
Total	1126	830	296	1.00	.50–.30
Resistant × Intermediate					
Perry × Illini	316	237	79	0.00	.99
Wabash × Chief	281	214	67	0.20	.70–.50
Wabash × Perry	172	135	37	1.12	.30–.20
Total	769	586	183	0.59	.50–.30
Intermediate × Intermediate					
Perry × Chief	258	0	258		
Susceptible × Intermediate					
Chief × Hawkeye	282	0	282		
Gibson × Perry	443	0	443		
Perry × Hawkeye	194	0	194		
Total	919	0	919		
Susceptible × Susceptible					
Gibson × Hawkeye	185	0	185		

dance of sporulation. Resistant varieties had only occasional spots, and these were usually small and nonsporulating.[2]

The present studies were made to determine 1) if resistance in different varieties is conditioned by the same gene, and 2) if modifying factors are involved in the intermediate reaction.

MATERIALS AND METHODS.—Wabash (resistant) × Lincoln (resistant) crosses were made in 1952; and various crosses were made in 1952 and 1953 between C1076 and C1079 (sister lines of experimental strains), Wabash, and Illini (resistant); Perry and Chief (intermediate); and Hawkeye and Gibson (susceptible). The F_2 plants were grown at Worthington, Indiana, in 1954 and 1955. A selected number of F_2 plants of each reaction type were advanced to F_3 plant rows to verify the F_2 segregation. Twenty to 25 seeds were planted in each F_3 plant row.

The susceptible variety Hawkeye was planted in rows adjacent to the F_2 and F_3 populations. Parent varieties were included in the tests. All plants were inoculated artificially 3 times at 2-week intervals each year. Only moderate infection resulted and it was impossible to distinguish between intermediate and highly susceptible parents and segregates. Therefore, segregates were classified only as resistant or susceptible.

EXPERIMENTAL RESULTS.—F_2 segregation of all crosses is shown in Table 1. Since the chi-square test indicated no significant difference between the progenies of each F_1 plant within crosses, the data for each cross are combined. Breeding behavior of F_3 progenies are shown in Table 2. The progenies from each cross within a reaction class behaved similarly, so the data for each reaction class are combined.

All F_2 progenies of resistant × resistant crosses were resistant and bred true resistant in F_3, indicating that resistance in these varieties is controlled by the same gene.

A satisfactory fit to a 3:1 ratio was obtained in the F_2 progenies of each resistant × susceptible and resistant × intermediate cross. The progenies of all resistant F_2 plants were in a ratio of 1 homozygous to 2 heterozygous resistant for each cross. The progenies of all susceptible F_2 plants tested were homozygous susceptible.

In the intermediate × intermediate, susceptible × intermediate, and susceptible × susceptible crosses, all the F_2 progenies were susceptible and all progenies of F_2 plants tested were homozygous susceptible.

TABLE 2.—*Breeding behavior of F_2 progenies of soybean crosses in 6 reaction classes to Cercospora sojina*

Reaction class	Number of F_2 plants tested	Reaction to Cercospora in F_2	Behavior of F_2 progenies	1:2 ratio χ^2	1:2 ratio P
Resistant × Resistant	57	Resistant	All resistant		
Resistant × Susceptible	29	Susceptible	All susceptible		
	83	Resistant	33 resistant		
			50 segregating	1.54	.30–.20
Resistant × Intermediate	19	Susceptible	All susceptible		
	35	Resistant	15 resistant		
			30 segregating	0.00	.99
Intermediate × Intermediate	88	Susceptible	All susceptible		
Susceptible × Intermediate	45	Susceptible	All susceptible		
Susceptible × Susceptible	5	Susceptible	All susceptible		

The breeding behavior of resistant × intermediate and intermediate × intermediate crosses indicates that the intermediate susceptibility of Chief and Perry is not conditioned by modifying factors acting on resistant genes. Because of the inability to differentiate between intermediate and highly susceptible reactions in this study, it could not be determined whether there was an intermediate gene at this locus or a modification of the gene for susceptibility.

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DISSEMINATION OF VENTURIA INAEQUALIS ASCOSPORES[1]

P. M. Miller and P. E. Waggoner

SUMMARY

When an extended rain began, the first maximum for air-borne ascospores of *Venturia inaequalis* (Cke.) Wint. was followed by a second maximum in 9–14 hours. The appearance of ascospores in the air ceased while about ¼ of the spores still remained in the asci, undoubtedly through an observed weakening of the discharge force. Some ascospores were air-borne during fair days at the height of the discharge season. The date of the first discharge was primarily affected by the winter weather, with some influence by spring weather. An estimate was made from climatological data of the probabilities that a given stage of tree growth will be found by the first ascospores.

Apple scab and its causal agent, *Venturia inaequalis* (Cke.) Wint., have been studied for many years by workers wherever apples grow. Several workers (1, 7, 9) studied the discharge and dissemination of *V. inaequalis* ascospores by exposing coated glass slides among apple trees or on apple leaves on the ground. Others (6, 11) drew spores into a trap in a stream of air, thus increasing the efficiency of trapping. Despite certain deficiencies in these methods, the major features of the discharge and dissemination of ascospores of *V. inaequalis* have been well delineated.

When an efficient volumetric spore trap became available (4), we began a re-examination of the factors affecting the discharge of *V. inaequalis* ascospores in the northeastern United States. A preliminary report has been presented (8). Hirst (5) recently employed the same trap in a 2-year study of *V. inaequalis* in England.

MATERIALS AND METHODS.—The method of spore trap operation and the counting of spores have been described (4). The orifice of the trap was 19 in. above the soil. A centrifugal fan drew air into the trap. The trap was operated from April 1 to June 4 (except April 6–7) in 1956, among 28 McIntosh apple trees that were 35 years old and 20 ft. tall and had been heavily infected in 1955. In 1957 the trap was operated from April 1 to June 14 (except May 15–17) among 4 similar McIntosh trees that had been heavily infected in 1956. An hourly record of ascospore concentration in the air was obtained during these 2 seasons.

The 2 seasons were markedly different in temperatures. Fig. 4 gives the temperature course during the cold spring of 1956 and the warmer spring of 1957. The points are the weekly mean temperatures for Mt. Carmel, the site of the trapping. A more informative view of the variation between the seasons is provided by Table 1, which gives the frequency of occurrence of months colder than the cold months or warmer than the warm months. For example, March–May 1956 was cold, only 3–12 per cent of these months in other years being colder. These frequencies are based on 30 years of data for Storrs, Connecticut: the monthly means were found to be normally distributed, their variances were calculated and the normal (Gaussian) curve used to estimate frequencies.

[1] Accepted for publication March 10, 1958.

TABLE 1.—*Frequency of months that are warmer than the warm months or colder than the cold months of 1955-56 and 1956-57* [a]

Oct. 1955—May 1956	Oct. 1956—May 1957
27% of the Octobers are warmer	39% of the Octobers are warmer
37% of the Novembers are colder	30% of the Novembers are warmer
2% of the Decembers are colder	16% of the Decembers are warmer
46% of the Januaries are warmer	3% of the Januaries are colder
14% of the Februaries are warmer	8% of the Februaries are warmer
7% of the Marches are colder	27% of the Marches are warmer
12% of the Aprils are colder	21% of the Aprils are warmer
3% of the Mays are colder	50% of the Mays are warmer

[a] These are based on records for 30 years at Storrs, Connecticut, about 70 miles northeast of Mt. Carmel, Connecticut, site of test.

RESULTS.—Except for occasional ascospores, the first discharge of 1956 started at 5 a.m. on April 26; the first ascosporic discharge of 1957 started at 4 p.m. on April 25—or 1 year later, less only 13 hours.

The highest concentration of ascospores per m^3 of air during each major period of discharge is shown in Fig. 1–2 (so-called "fair weather discharges," discussed below, are not shown). In 1956 this maximum concentration per period rose steadily from the first discharge to a maximum concentration of 21,600 ascospores per m^3, on May 12, and thereafter decreased; on June 2–3 it was only 200 spores per m^3 even though the overwintered leaves were wet over 48 hours. In 1957 there were fewer discharge periods, because of fewer wetting periods. Because of a spore trap failure, we obtained no data during the wetting period of May 15–17. In 1956 these dates had been near the period of maximum rate of discharge. The counts obtained in 1957 for discharges before and after May 15–17 followed essentially the same pattern as in 1956.

Discharge started within an hour after the start of rain, regardless of temperature. A second peak of discharge occurred several hours after the first peak if rain continued more than 10 hours. Fig. 3 shows 2 examples of these double peaks of ascospore concen-

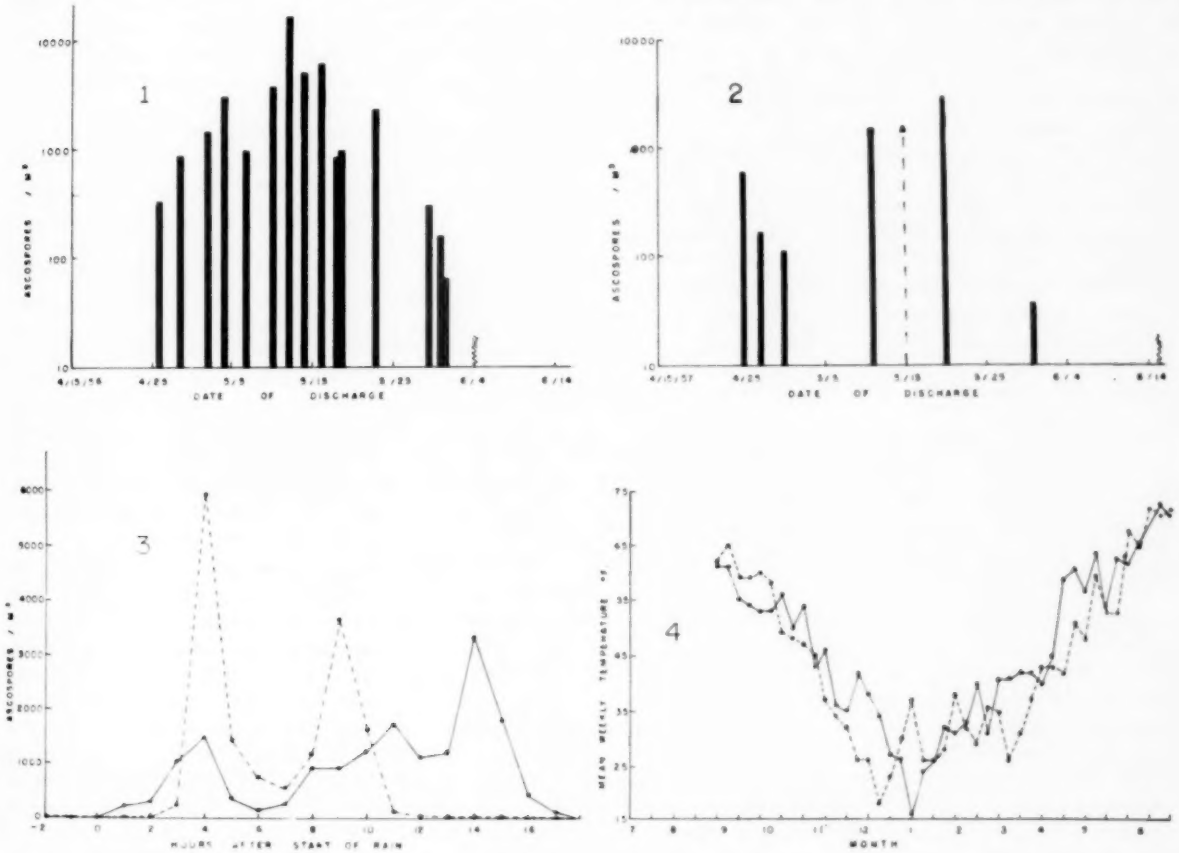

FIG. 1–4. Fig. 1. The highest concentration of air-borne ascospores obtained during each major period of discharge in 1956. The ragged vertical line indicates cessation of trapping.—Fig. 2. Concentrations of ascospores in 1957, obtained as in Fig. 1. The dashed vertical line is the estimated concentration during a rain when the trap was not operating.—Fig. 3. The concentrations of air-borne ascospores at hourly intervals before and after the start of 2 rains in 1956.—Fig. 4. The average weekly temperatures at Mt. Carmel in 1955–56 (dashed line) and in 1956–57 (solid line).

tration during 2 discharge cycles in 1956. This phenomenon was frequent in 1956 as well as in 1957, when discharge was less abundant.

The trap was not operated after June 4, 1956, because low spore counts during the previous 3-day wetting period indicated that ascospore discharge was at an end. In 1957 the trap was operated until June 14 to observe any lingering ascospores. Since no wetting period had occurred in the preceding week, all areas within 40 ft. of the trap were soaked for 5 hours with 1 in. of water/hour. Not a single ascospore was found on the slide, and operation of the trap was discontinued.

Periodic examination of the development of the fungus in the overwintering leaves was made after April 1. The asci and ascospores were mature only a short time before the date of the initial discharge. E. M. Stoddard called our attention to the discontinuous nature of the discharge of ascospores from perithecia, as shown in water on slides. The repeated puffs undoubtedly were caused by step-wise maturation of asci. The perithecia still contained 25–35 per cent of their ascospores at the beginning of the wetting period of June 1–3, 1956. Yet very few spores were caught during this period in the trap 19 in. above these leaves. Ascospores merely oozed, or were ejected weakly, from the asci into water on a glass slide, contrasting with the typically forceful and sharp ejection observed during the early and middle portions of the discharge season.

Although rain always accompanied the heavy discharges, very light and short discharges of less than 150 ascospores per m^3 occurred in fair weather on May 5, 15, 19, and 24, 1956. These "fair weather discharges" usually occurred around noon, lasted only 1–3 hours, followed nights with relative humidity above 95 per cent, and occurred in the middle portion of the discharge season, when the perithecia would have a maximum content of mature ascospores. The fair weather discharge of May 19, 1956, at 5–11 a.m., was an exception.

We also observed the unfolding of the apple buds. A difference between the stages of development of the fruit buds in 1956 and 1957 was evident at the time of initial ascospore discharge: in 1956, early delayed dormant (with full bloom on May 19); in 1957, prepink (full bloom on May 1).

Discussion.—Fancier tools do not make a better craftsman, and the use of a more refined spore trap than that of Childs (3) or Keitt and Jones (6) did not provide data that would justify challenging their findings. Our results also conform very closely with those of Schneiderhan and Fromme (9) and Wilson (10). The contributions of our study are the observation of repeated maxima of spores air-borne during a single storm, observation of "fair weather" discharges, and the showing of exhaustion of the discharge mechanism before exhaustion of the spore supply.

Environmental factors regulate the maturation of the 2 organisms, fungus and tree. Weather records were examined for an explanation of the relatively late bloom of 1956 and the constancy of the time of first ascospore discharge. Precipitation was—as usual in the Northeast—adequate to saturate the fallen leaves during the winter. In both seasons, snow covered the leaves most of the period January 1–February 15; in 1956 snow cover was frequent until mid-April. Thus precipitation and snow cover were similar enough not to change the date of first discharge (10).

Explanation of the contrasting behavior of fungus and host must lie in the temperature (Table 1 and Fig. 4). Wilson (10) has said that maturation of the perithecia of *V. inaequalis* is affected by conditions throughout its saprophytic life, even in winter. In fact, the total periods of cold and warm weather in the autumns and winters of 1955–56 and 1956–57 were not greatly different. Evidently, the cold weather in the spring of 1956 arrived too late to alter greatly the ripening of the asci. Hence, given rain at nearly the same time in the 2 years, the first discharge occurred at nearly the same time. These observations suggest that spring temperatures are relatively unimportant in discharge but have greater importance in infection.

The synchronous discharge observed in the 2 years was doubtless by chance, for variation has been observed (1, 6, 9, 11). The variation has been attributed to alternate wetting and drying of fallen leaves (6) as well as to temperature. If both factors are important, then variation in the northeastern United States should be less than in some other regions, because our relatively low temperatures and adequate precipitation in fall and winter generally produce a saturated litter on the soil. Thus, one variable is removed in that region.

Bradford (2) has attributed variation in the time of blooming to variations in "degree-days." These degree-days were calculated by subtracting 43°F from each daily maximum temperature, omitting negative values, and summing the positive values from March 15 to date of full bloom. In fact, this sum was 706F°-days in 1956 and 723F°-days in 1957, very close agreement with Bradford's hypothesis. Thus, late blooming in 1956 was due to cold spring temperatures in 1956 (Table 1). The small difference could be due to the delaying of the 1957 bloom by the cold spring of 1956, as suggested by Lake (7), or to the choice of beginning or ending date for calculating F°-days.

We can make for the northeastern United States an estimate of the probability that a given stage of tree growth will be found by the first ascospores. The autumn and winter temperatures, viewed as a whole, were near normal in 1956 and 1957, cold spells canceling out warm ones. Therefore, about half of the autumn-winter seasons can be expected to resemble 1956 and 1957 closely; consequently, the first spore discharge will be within, say, 5 days of April 25 half of the time. On the other hand, weather as cold as March–April, 1956, will occur less than 10 per cent of the time; consequently, blooming as late as May 19 will occur less than one fourth of the time. Therefore, blooming must occur between May 1 and 19 about 7 out of 10 years. The events of discharge and blooming evidently being independent, one can estimate from

the above limits the joint probabilities of 2 simultaneous events. For example, first discharge and full bloom will both occur in the first 10 days of May some 5–10 per cent of the time.

Inability to trap spores in late spring has long been known and attributed to the depletion of the contents of the perithecia. The weak expulsion of the spores in June, although one fourth remained in the asci, leads to a new interpretation. The lack of air-borne ascospores must be due not to depletion of the supply, but rather to poor discharge that was too weak to get the ascospores air-borne.

The fair weather discharges found in these studies are of doubtful importance to a fruit grower who follows a protective spray program. They might become an important factor if he applies only eradicant sprays on the tree after infection periods; in this system he would not spray to kill the spores discharged in fair weather. Ascospores germinated well even after 6 days' exposure to full sunlight on the upper surfaces of leaves of potted plants in a greenhouse (6). Therefore, ascospores discharged in early afternoon should be able to survive until darkness, when they might germinate in the dew and infect the host. Since these discharges were very light, they are important, not because they produce extensive infections but because the infections can serve as sources of conidia for secondary infections after the supply of ascospores is depleted. Another possible fair-weather discharge is indicated in data of Childs (3) as occurring on a clear day preceded and followed by 2 clear days, just prior to the maximum discharges of the season.

Hirst (5) never observed 2 discharge peaks within 24 hours. Several times in 1956 and 1957 more than 1 peak of discharge within 24 hours and even within 12 hours (Fig. 3) was observed at Mt. Carmel. The first peak of discharge is the spores matured before the start of the wetting period. The succeeding peaks arise from step-wise maturation of asci or spores such as observed with the microscope.

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PARASITISM OF TWO SPECIES OF DAGGER NEMATODES (XIPHINEMA AMERICANUM AND X. CHAMBERSI) TO STRAWBERRY[1]

Vernon G. Perry[2]

SUMMARY

Two species of dagger nematodes, *Xiphinema americanum* and *X. chambersi*, are found associated with a decline of strawberry in Wisconsin. *X. americanum* is widespread and is found on several crop plants, whereas *X. chambersi* has been found in 1 strawberry field only. Inoculation experiments showed that these 2 nematodes can cause sunken, reddish-brown lesions on strawberry roots that progress to an eventual blackening of the entire root system. It is suggested that other organisms follow the nematodes and bring about eventual destruction of the roots. Strawberry plants inoculated with either species of dagger nematodes or with northern root-knot nematodes did not grow as well as did the control plants and had smaller root systems and more root discoloration.

Many strawberry plantings in Wisconsin show an unexplained but definite decline, with lowered production and even death of plants. Search for a satisfactory explanation of this condition led to investigation of the nematode fauna of declining fields. The plant parasitic nematode most commonly found in a preliminary survey was the American dagger nematode, *Xiphinema americanum* Cobb, 1913 (4, 7). Another dagger nematode, *X. chambersi* Thorne, 1939, was found in 1 field. In addition, 3 species of root-lesion, or meadow, nematodes, *Pratylenchus penetrans* (Cobb, 1917) Sher & Allen, 1953, *P. pratensis* (de Man, 1880) Filipjev, 1934, and *P. minyus* Sher & Allen, 1953, and northern root-knot nematode, *Meloidogyne hapla* Chitwood, 1949, were often found.

Previous efforts by the author and others (6) to establish populations of *Xiphinema americanum* in the greenhouse resulted in failure, but Christie (2), Cobb (4), Thorne (7), and other nematologists have recognized *X. americanum* as a plant parasite. Direct evidence of pathogenicity is presented in an unpublished thesis by White (9), who established populations on shortleaf pine seedlings and found that definite stunting occurred. Christie (2) reported that *X. americanum* "feeds at root tips and along the sides of roots, devitalizing the tips and causing very extensive necrosis." Chapman (1) found *X. americanum* in 36 of 50 strawberry fields sampled in Kentucky, and concluded that it "should be regarded as a potentially important pathogen of this crop in Kentucky."

The experiment reported herein was started to determine the pathogenic nature to strawberry of *X. americanum* and *X. chambersi*.

EXPERIMENTAL PROCEDURE.—Soil containing *X. americanum* was collected from a strawberry field and from a raspberry planting, and soil containing *X. chambersi* was collected from a strawberry field. These collections also contained a variety of other nematode species. The nematodes were extracted from the soil by the combination sieving-Baermann-funnel technique developed by Christie and Perry (3). In this procedure, soil washings were passed through superimposed 20-, 100-, and 200-mesh sieves, and the retained debris was rinsed until clear. Material caught on the 200-mesh sieve was placed on a muslin screen in the Baermann funnel. The next day the nematodes were drawn off in a few ml of water and used to infest pots 1b and 2b, as "miscellaneous nematodes" (Table 1). This collection consisted of about 20 species, including predators, saprophages, fungal feeders, ectoparasitic species, and endoparasitic species.

In the sieving process, most of the dagger nematodes were retained by the 100-mesh sieve. The nematodes described as "picked" specimens (Table 1) were obtained by selecting individual nematodes from the material removed by a Baermann funnel and placing them in distilled water. This gave a Xiphinema culture entirely free of other nematodes and most other organisms. The nematodes designated "mass" were obtained by placing the material from the 100-mesh sieve in Baermann funnels and using the nematodes extracted from the funnels. These mass collections contained other kinds of nematodes besides species of Xiphinema, but those with more than a few specimens of other species were discarded.

Wisconsin Plainfield sandy loam from a raspberry planting was autoclaved 4 hours at a pressure of 10–12 lb. for use as potting soil. Strawberry plants produced from runners rooted in steamed soil were supplied by F. A. Gilbert, Horticulture Department, University of Wisconsin.

The inoculation procedure was as follows: 1) Nematodes were collected from soil by the techniques described above. The funnels were set up during an afternoon and the specimens removed the next morning and used the same day, thus remaining in water for 24 hours or less. 2) Roots and tops of the strawberry plants were pruned extensively to leave only 1–2 leaves plus the bud and 3–4 main roots. The plants were then placed in 6-in. pots, and the roots were partly covered with steamed soil. 3) The water suspension of nematodes was poured directly onto the roots, and the pots were filled with steamed soil. 4) The pots were thoroughly watered, and placed on inverted saucers to prevent

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possible contamination by nematodes from the benches.

The experiment, conducted in a greenhouse, was started May 1, 1956, and continued as shown in Table 1. The summer of 1956 was mostly cool and moist, which favored culturing nematodes under greenhouse conditions. Temperature of the soil in the pots, checked periodically, was not observed to be above 85°F at any time; from May through September it was mostly 70–80°F; after October 1, the range was 65–70°. Untreated water from Lake Mendota was used for watering. Extreme care was taken to keep the soil moist at all times, since it was thought that even a mild drying might kill the nematodes. No fertilizer was added during the course of the experiment.

On July 10, plants and soil from pots 4a and 4b were removed for examination. The plants from the remaining pots were removed, examined, photographed, and preserved on November 29, 1956.

RESULTS.—No difference was observed between the plants in pot 4a, which received a mass inoculation of *X. americanum*, and pot 4b, the corresponding control; these were examined a little less than 2 months after the experiment was started. Only a few dagger nematodes were found in pot 4a, and none were found in pot 4b.

When part of the soil from each of the remaining pots was washed and examined on November 29, 1956 (i.e. 6–7 months after the experiment was started), many specimens of *X. americanum* were found in all the pots that had received picked specimens or the mass inoculum of this species. A few non-parasitic nematodes were found, but these were found in the control pots also.

All control plants had an abundance of white, healthy roots, with extensive branch and feeder rootlets (Fig. 1.D). Some of the older, centrally located roots were discolored, a condition presumed to be normal for strawberry. Both top growth and root production were surprisingly consistent on all the control plants, even though 3 different strawberry varieties were used (Table 1).

Moderate but not distinctive root injury was observed on all plants inoculated with *X. americanum*; extent of injury could not be correlated with the number of specimens used. Root color ranged from the normal white of new, healthy roots to the tan and black of the older, more severely injured ones (Fig. 1.D). The root systems as a whole were almost as extensive as those of the controls, perhaps indicating that the injury was late in occurring. The small number of nematodes in pot 4a after 2 months' exposure might also indicate that the main attack in other pots of the experiment occurred late, after the roots had formed.

Somewhat sunken, reddish-brown lesions occurred on the newer roots, both at obviously blinded tips and elsewhere all along the surface from the tip to the point of origin. These lesions ranged from small, mottled ones of varying shape to others that extended the entire circumference of the root. This type of injury was reproduced by placing new plants in pots 1a and 2a for a period of 2 weeks immediately after removal of the first test plants.

The injury produced by *X. chambersi* (Fig. 1.A,B,C,) was moderate to severe, and proportionate to the number of nematodes used as inoculum. Root symptoms, again, were not very distinctive, but were more severe than those produced by *X. americanum*. The root systems of inoculated plants were extensively discolored. For the most part the discoloration was black, but reddish-brown lesions similar to those produced by *X. americanum* were observed. Note in Fig. 1 that the only healthy-appearing roots on the inoculated plants were those produced last. Unfortunately, pot 6a was destroyed before completion of the experiment; however, the plant was observed to be stunted somewhat less than the plant in pot 6d, which received about 2000 specimens.

TABLE 1.—*Dates of inoculation, strawberry varieties inoculated, and source and kind of inoculum used to test pathogenicity of dagger nematodes, 1956*

Strawberry variety	Date of planting and inoculation	Pot No.	Source of nematodes	Inoculum	
				Kind	No. specimens added
Dunlap	May 1	1a	Strawberry field	*X. americanum*	Picked 400
		1b	Strawberry field	Miscellaneous	—
		1c		None	
Robinson	May 3	2a	Strawberry field	*X. americanum*	Picked 600
		2b	Strawberry field	Miscellaneous	—
		2c	Raspberry planting	*X. americanum*	Mass 1000
		2d		None	
Catskill	May 9	3a	Raspberry planting	*X. americanum*	Mass 1000
		3b	Strawberry field	*X. americanum*	Mass 1000
		3c		None	
Robinson	May 16	4a	Raspberry planting	*X. americanum*	Mass 1000
		4b		None	
Dunlap	May 17	5a	Raspberry planting	*X. americanum*	Mass 1000
		5b		None	
Robinson	June 5	6a	Strawberry field	*X. chambersi*	Picked 1000
		6b	Strawberry field	*X. chambersi*	Picked 250
		6c	Strawberry field	*X. chambersi*	Mass 500
		6d	Strawberry field	*X. chambersi*	Mass 2000
		6e		None	

Specimens of *X. chambersi* were scarce at the end of the experiment, possibly because there were only a few healthy roots for them to feed on. As in the pots infested with *X. americanum*, only a few other nematodes were present on completion of the experiment.

The plants in pots 1b and 2b (Table 1), which were inoculated with miscellaneous nematodes, were severely stunted almost from the beginning of the experiment; and, upon removal at the end of the experiment, the root systems were found to be unhealthy and abnormal. Examinations revealed an abundance of the root-knot nematode, *Meloidogyne hapla* Chitwood 1949, and a few nonparasitic species. Obviously the "miscellaneous nematodes" used for inoculum contained larvae of this root-knot species. The roots were black, and bore small galls and club-shaped, blinded tips.

Discussion and conclusions.—The symptoms of dagger nematode injury produced experimentally are like those observed on commercial plantings of strawberries in Wisconsin where these nematodes are present. In both cases, the affected roots first have small, indeterminate, reddish-brown, sunken lesions, which may occur on the roots at any point. Finally, the roots become dark-brown to black. Field decline attributed to these nematodes is apparently rather slow, since production during the first season, and perhaps the second, may not be materially affected. Wisconsin strawberry growers are well aware that, in most cases, it is not profitable to maintain plantings for longer than 2–3 seasons of production.

The experiments indicate that dagger nematodes cause injury that results in the eventual blackening of strawberry roots. A blackening of the roots was also observed in association with injury caused by the northern root-knot nematode.

Undoubtedly, some mechanical damage to root cells is produced by the stylets of dagger nematodes in the process of feeding, and it is highly probable that additional damage is caused by the injection into the plant of secretions from their oesophageal glands. This injury

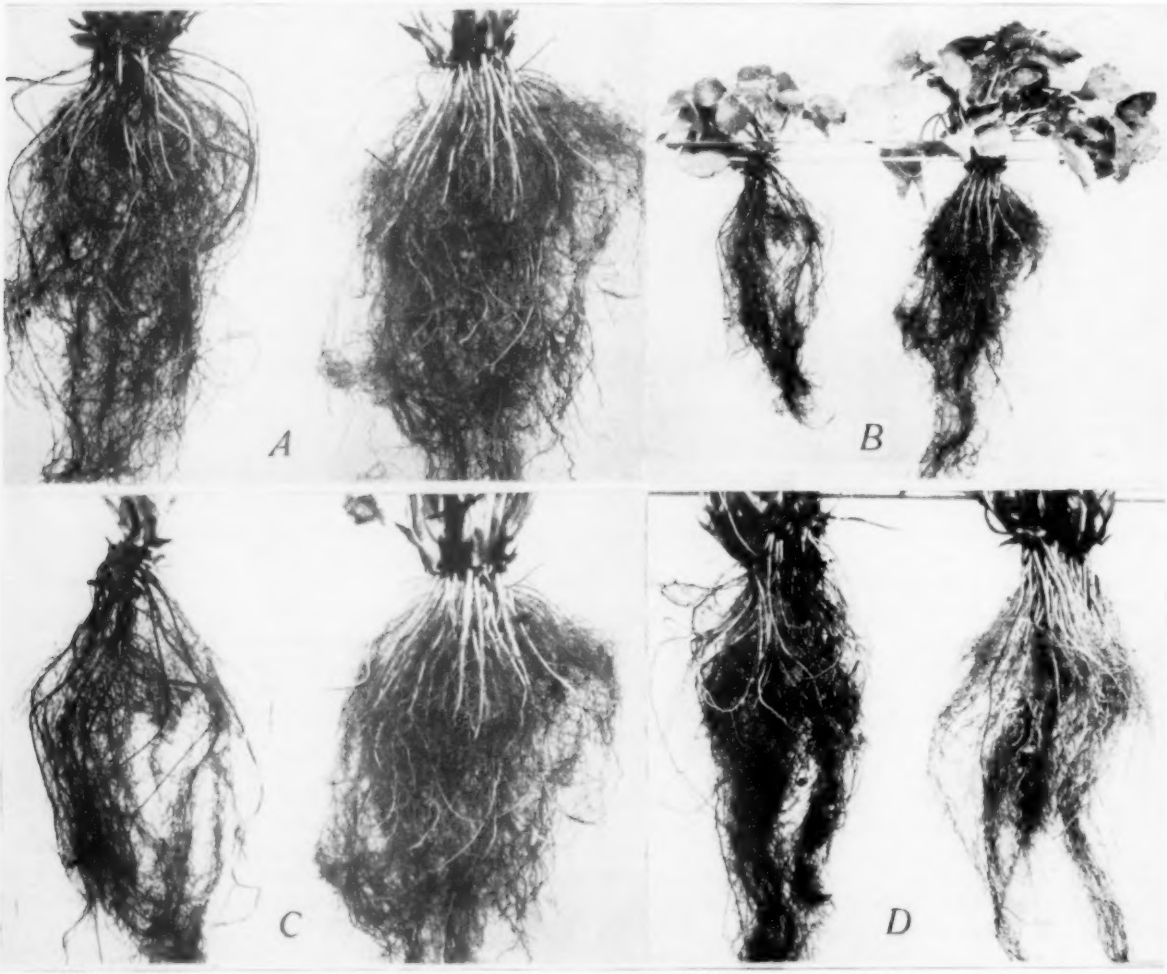

Fig. 1. Strawberry roots injured by dagger nematodes and comparable noninoculated roots. A) Left, from a pot infested with 250 picked specimens of *Xiphinema chambersi*. Right, from control pot. B, C) Left, from a pot infested with "mass populations" of *X. chambersi*. Right, from control pot. D) Left, from a pot infested with 400 picked specimens of *X. americanum*. Right, from control pot.

might be the cause of the sunken reddish-brown lesions observed in the experimental pots and in the field. It is suggested that these primary lesions are then invaded by bacteria, fungi, and other nematodes, which complete the discoloration and eventual destruction of the roots.

The lesser degree of injury brought about by *X. americanum*, as compared with the injury caused by *X. chambersi* and root-knot nematodes, is considered due to the relative time of attack by the nematodes. The experiments indicated that the major injury by *X. americanum* occurred after 2 months' exposure, probably because of poor survival of the specimens used as inoculum. Comparative field symptoms indicate that injury by *X. americanum* may be just as severe as that caused by *M. hapla* or *X. chambersi*.

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PATHOGENICITY OF FUSARIUM ROSEUM TO CARNATION AND TO WHEAT[1]

James Tammen [2]

SUMMARY

Sixty-two clones of *Fusarium roseum* were studied in a series of 5 cross-pathogenicity tests to determine whether the clones that incite a root, crown, and stem rot disease of the perpetual flowering carnation were pathogenically distinct from those of *F. roseum* f. *cerealis*, which incite a seedling blight and root or foot rot of cereals. Carnation clones of the fungus pathogenic to carnation produced the seedling blight and root or foot rot of wheat; cereal clones pathogenic to wheat produced the root, crown, and stem rot of carnation. In addition, clones originally isolated from several other hosts were found to be pathogenic to both carnation and wheat. It is concluded that pathogenic clones of *F. roseum*, irrespective of the original host from which they were isolated, are not pathogenically specialized in respect to the tested host plants, and that the carnation pathogen is *F. roseum* f. *cerealis*.

One of the most serious diseases of the perpetual flowering carnation (*Dianthus caryophyllus* Linn.) in the United States (1, 2, 3) is a root, crown, and stem rot incited by members of the species *Fusarium roseum* (Lk.) Snyd. & Hans. A root or foot rot and seedling blight of cereals incited by this fungus has long been recognized (7).

Biologic specialization of isolates of *F. roseum* associated with cereal root rots was demonstrated by Oswald (10, 11), who found that certain of these isolates were pathogenic to members of the Gramineae but that others were not. Snyder and Hansen, in their revision of the genus *Fusarium* (13), placed the cereal pathogens of *F. roseum* in the forma specialis *cerealis* (Cke.) Snyd. & Hans. to distinguish them from the saprophytic members of the species. Though they noted that certain of the Fusaria grouped under this species had been reported to attack hosts other than cereals and stated that new forms should be established for them if they were found to be biologically distinct from *F. roseum* f. *cerealis*, the relationship between the carnation pathogen and the cereal pathogen has not been determined. Evidence indicative of this relationship, however, was reported by Wickens (17), who found that strains of the fungus isolated from oats and wheat were slightly pathogenic to carnation.

The primary purpose of this study was to determine whether clones of *F. roseum* pathogenic to carnation were pathogenically distinct from those of *F. roseum* f. *cerealis*.

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MATERIALS AND METHODS.—In order to demonstrate pathogenic specialization in *F. roseum*, cross-pathogenicity tests were made. These involved the testing of the pathogenicity of a given isolate or clone[3] to carnation and cereals under identical environmental conditions. Whether that clone was pathogenic to one or both plants was determined by symptoms.

The environmental conditions were equalized by planting 20 carnation cuttings in one half of a small flat and 20 cereal seeds in the other half, each equidistantly spaced and planted to the same depth in sterile moist sand.

Wheat (var. White Federation '38) was selected for use as the cereal host because it had been found to be the cereal crop most susceptible to attack by the fungus (10, 11). Prior to planting, the wheat seed was treated with Arasan (50% thiram, which is bis(dimethylthiocarbamoyl) disulfide), to destroy surface contaminants (16). Because of this treatment, inoculation was delayed until after the seed had germinated.

Vegetative cuttings of carnation were chosen for use because preliminary tests had shown that they were readily attacked by clones of *F. roseum* and the extent of the invasion could be measured and classified. The varieties used depended on their availability, but all cuttings in any 1 test were of the same variety. The cuttings were indexed by culturing (15) to eliminate those infected with *Fusarium oxysporum* f. *dianthi* (Prill. & Del.) Snyd. & Hans. (4) and *Pseudomonas caryophylli* Burk. (5). At planting, they were cut just below the node and dipped in a commercial talc-hormone preparation.[4] The cuttings were planted and inoculated after the wheat seed had germinated.

To obtain representative isolates of *F. roseum* from carnation, a survey (1950–1953) was made of the major carnation-growing areas of California. Numerous isolates were obtained from cuttings, rooted cuttings, and young plants, and from older plants showing the various phases of the disease as described by Wickens (17). Twenty-eight of these isolates, representing the several cultural types (11), were selected for testing. Clones originally isolated from cereals and other hosts (Table 1) were obtained from a collection gathered by J. W. Oswald and W. C. Snyder.

Each clone tested was single-spored (8). One single-spore culture, identical to or closely resembling the "wild" or "natural" type, was selected and increased by mass transfer on 20-ml potato dextrose agar slants. From these, spore suspensions (100 ml) were prepared by washing the surface of the colony with sterile distilled water. The spore concentration of each clone was approximately equalized by examining a drop of the suspension with the aid of a microscope and altering the dilution to achieve uniformity.

Each flat was infested by pouring 2 such spore suspensions over the surface of the sand, followed by watering to distribute the inoculum throughout the medium.

Clone No. 33, isolated from oats, was used in 4 of the tests, and clone No. 72, isolated from carnation, was used in all 5 tests; this made it possible to relate one test to another. These clones were termed "standards."

During the tests, the plants were watered weekly with Hoagland's nutrient solution No. 1 (9).

Four to 5 weeks after the addition of the spore suspension, the plants were dug, washed, and grouped into classes from 0 to 5, depending on severity of disease symptoms. The classes used as a basis for mean disease rating (MDR) of carnation cuttings were as follows: 0, cuttings rooted, with no symptoms on either the aboveground or belowground parts; 1, cuttings not rooted and showing evidence (trace) of brown basal rot, slight wilting, graying, and necrosis of leaves; 2, definite evidence of a brown basal rot (trace to ½ in.), tops wilted and showing definite leaf graying, burning, and die-back; 3, brown basal rot extending ½ in.–1 in. up the stem, top symptoms as in class 2; 4, brown basal rot extending 1 in. to 1½ in. up the stem, top symptoms as in class 2; 5, brown basal rot extending over 1½ in. up the stem, cutting dead at termination of test.

The classes used as a basis for mean disease rating of cereal seedlings were those of Oswald (11) except that top symptoms were not utilized. These were: 0, seedlings healthy; 1, brown discoloration and lesions on the coleoptile, but not extending into the sub-crown internode, crown, or culm; 2, brown discoloration and lesions extending into the sub-crown internode, crown, and culm; 3, coleoptile, sub-crown internode, crown, and lower culm severely rotted; 4, plants nearly dead at harvest; 5, plants dead at harvest.

To determine the pathogenicity of a given clone to the carnation and wheat hosts, a mean disease rating was calculated by multiplying the number of plants in each class by the class number, summing the products, and dividing by the total number of plants tested. An MDR of 0.0 would indicate that all plants were healthy, and an MDR of 5.0 that all plants were killed.

Reisolations from infected plants, after grading, indicated that no intermixing of clones had occurred between test flats.

RESULTS.—Sixty-two clones of *F. roseum* were assayed in 5 separate tests (Tables 1 and 2). Many of those collected from cereals were found to be pathogenic to wheat seedlings and carnation cuttings; many of the clones collected from carnation were found to be pathogenic to carnation cuttings and wheat seedlings. Some of the clones originally isolated from other plants attacked both carnation and wheat.

Differences in MDR occurred on testing the 2 hosts with a particular clone. In a few cases, the MDR obtained from wheat was higher than that from carnation. In others, the MDR obtained from carnation was higher than that from wheat. These differences were relative in degree, independent of original host from which the clone was isolated, and hence not indicative of pathogenic specialization in *Fusarium roseum*.

[3] As used here, the term "clone" designates a given isolate obtained in pure culture and so maintained by the single-spore method of Hansen and Smith (8).

[4] Hormodin A, No. 1 (2 mg indole butyric acid/g talc) Merck and Company, Inc. Rahway, New Jersey.

Because the carnation clones of *F. roseum* pathogenic to carnation will incite the seedling blight and root or foot rot of wheat, and because the cereal clones pathogenic to wheat will incite the root, crown, and stem rot of carnation, it is concluded that pathogenic specialization in regard to these 2 hosts does not occur. That clones of *F. roseum* may not show any host specificity is suggested by the limited tests wherein clones isolated from hosts other than carnation and cereals attacked both carnation and wheat.

Each clone tested showed some degree of pathogenicity to the test plants, but that degree varied from essentially none to moderate or severe. Thus, certain clones of *F. roseum* exhibited a very low degree of pathogenicity to the 2 types of test plants. These clones probably are incapable of causing economic loss, and for all practical purposes may be considered saprophytic. Other clones of the fungus exhibited a moderate degree of pathogenicity, and still others were highly pathogenic.

DISCUSSION.—In view of the fact that isolates of *F. roseum* will attack at least 2 widely divergent hosts, one might question the retention of the forma specialis *F. roseum* f. *cerealis*. In their conception of the form, Snyder and Hansen (12, 13) stated that it should be established below the species level to indicate either relative host specificity or pathogenicity. Since certain members of *F. roseum* are considered to be saprophytic, it is concluded, in keeping with the above concept, that the form be retained to separate the nonpathogenic members from those that are pathogenic, even though the line between saprogenicity and pathogenicity, particularly when carnation is the host, may not always be clear. Since *F. roseum* f. *cerealis* has long been identi-

TABLE 1.—*Mean disease ratings resulting from cross-pathogenicity tests on carnation and wheat[a] with clones of Fusarium roseum obtained from cereals and other hosts*

Test no.[b]	Original host	MDR[c] Carnation	MDR[c] Wheat
1	Cereal	0.6	0.5
		0.9	1.7
		1.1	0.7
		1.3	1.8
		1.3	2.7
		1.4	1.6
		1.5	1.5
		1.7	1.6
		1.8	1.7
		2.0	0.5
		2.3[d]	1.4[d]
2	Cereal	1.1	0.2
		1.4	0.5
		1.4	0.4
		1.4	0.1
		1.5	0.2
		3.4[d]	2.4[d]
		4.0	2.5
3	Cereal	1.4	0.7
		2.4[d]	1.9[d]
4	Cereal	3.0	1.0
		4.8	1.9
		4.8[d]	2.1[d]
2	Artichoke	1.6	0.6
	Sugar beet	2.9	2.1
3	Celery	1.6	0.1
4	Lettuce	2.0	0.1
	Heather	3.9	0.5
	Snapdragon	3.9	1.3
	Bean	4.8	1.9
	Radish	0.2	0.9
	Alfalfa	2.0	1.6
		2.4	1.8

TABLE 2.—*Mean disease ratings resulting from cross-pathogenicity tests on carnation and wheat[a] with clones of Fusarium roseum obtained from carnation*

Test no.[b]	MDR[c] Carnation	MDR[c] Wheat
1	1.7	1.8
	1.8	0.6
	1.8	1.3
	1.8	1.2
	1.9[e]	1.6[e]
	3.1	1.6
2	1.5	0.3
	1.6	1.7
	1.9	1.4
	2.5	2.2
	2.6	1.9
	3.2	1.8
	4.1	2.1
	4.2	2.9
	4.3[e]	2.7[e]
3	0.9	0.8
	1.1	0.8
	1.2	0.3
	1.3	0.8
	1.3	0.6
	2.9	2.2
	3.1	1.3
	3.3	1.7
	3.6	1.7
	3.6	1.4
	3.7	1.9
	3.8	1.9
	4.0[e]	1.6[e]
	4.4	2.0
	2.6	1.5
4	3.8	1.2
	4.9[e]	1.6[e]
5	2.0	0.5
	2.5	1.3
	3.1	1.9
	3.7[e]	2.3[e]

[a] 20 carnation cuttings planted in 1 half and 20 cereal seedlings planted in the other half of a small flat; each inoculated with a single clone of *F. roseum*.
[b] Test number 1, etc., of Table 1 and test number 1, etc., of Table 2 were made concurrently.
[c] An MDR of 0.0 indicates no disease; an MDR of 5.0 indicates that all plants were dead at termination of the test.
[d] Standard clone No. 33.
[e] Standard clone No. 72.

fied with a disease of cereal crops (6), it is further concluded that the present form name be retained, and that its description be emended from one indicating specificity (13) to one indicating pathogenicity.

The description of the form is here emended so that *Fusarium roseum* f. *cerealis* pathogenic to Gramineae will now read *Fusarium roseum* f. *cerealis* pathogenic to Gramineae and *Dianthus* species.

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A COLOR TEST FOR EXOCORTIS INFECTION IN PONCIRUS TRIFOLIATA [1]

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SUMMARY

Ray cells in the bark of *Poncirus trifoliata* (L.) Raff. infected with exocortis virus are frequently atypical in unfixed, unstained sections, appearing darker (more opaque) than adjacent cells. The contents of these atypical cells react with aldehyde-coupling reagents such as phloroglucinol-HCl and others, giving characteristically-colored products that are clearly visible in cross sections of bark under the microscope. This reaction is useful as an indication of exocortis virus infection in *P. trifoliata* before the onset of bark scaling. This reaction was not found in unbudded *P. trifoliata* trees of various ages presumably free of exocortis virus or in *P. trifoliata* rootstocks known to be free of the virus. The presence of other viruses (psorosis, xyloporosis, and tristeza) in the top, and presumably also in the *P. trifoliata* stock, in addition to exocortis virus, appears to have no effect on the reaction. In 98.6 per cent of the cases tested, the color reaction correlated with the presence of the exocortis virus in *P. trifoliata* infected 2 years or longer.

The name "scaly butt" is preferred to exocortis because the virus nature of the disease was first demonstrated under that name by Benton et al (1, 2, 3); but exocortis is used hereinafter because of its wide use. Benton et al found bark scaling to be the definitive symptom of exocortis virus infection in *Poncirus trifoliata* (L.) Raff. They reported the usual incubation period to be 4–8 years. The length of the incubation period and the apparent absence of other reliable symptoms have seriously retarded the indexing of commercial varieties of citrus for freedom from exocortis virus.

Indexing for exocortis was not commenced as part of the Florida Citrus Budwood Program until 1955, because suitable seedlings of *P. trifoliata* were not available, but a more important delaying factor was the 8 years required for proof of non-infection. Consequently, under the present indexing procedure no candidate tree of the Florida program can be pronounced free of exocortis virus before 1963. This situa-

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tion presents problems that could lead to compromising the original intent of the Program, or threaten its existence. Therefore a search was undertaken for more precocious symptoms of exocortis virus infection. Certain anatomical abnormalities (6) previously noted in exocortis-affected *P. trifoliata* were investigated.

MATERIALS AND METHODS.—Attention was concentrated on *P. trifoliata* for several reasons: a) by definition, exocortis (scaly butt) is a disease of *P. trifoliata* (1); b) the Florida Citrus Budwood Program specifies *P. trifoliata* as the test plant for exocortis virus infection, and all trees currently under test are indexed on it; c) only *P. trifoliata*-rooted trees were available in sufficient numbers for experimental purposes;[5] and d) the exocortis-type scaling exhibited by certain citranges (4, 10), lemons (2), and limes (10) may or may not be caused by the exocortis virus, a point not established.

Rusk citrange (*Citrus sinensis* × *P. trifoliata*), Rangpur lime (*C. aurantifolia*), Cleopatra mandarin (*C. reticulata*), sweet orange (*C. sinensis*), and sour orange (*C. aurantium*) rootstocks were examined to a limited extent.

Initially, specimens of wood were collected with bark attached because interest was primarily in abnormalities of the xylem. Later, when it was learned that exocortis infection could be diagnosed from bark alone, wood specimens were omitted. The size of the bark specimen was determined largely by the size of the tree, but a piece ¾ in. long by ¼ in. wide bisected by the bud union was considered the minimum for routine examination: smaller specimens were difficult to handle in sectioning. Immediately after excision, bark pieces were stored in formaldehyde-acetic acid-alcohol solution No. 2 (11) until prepared for examination. For microchemical tests, sections of fresh, unfixed bark were used.

Transverse and longitudinal sections of wood with attached bark, and of detached bark, were cut with a Stanley No. 60½ block plane held in the hand. The blade of this plane cuts at an angle 14° to horizontal, several degrees flatter than other models (15). With the blade ground and honed to razor sharpness, this type of plane proved better and faster than a sliding microtome for sectioning wood and bark of citrus. It cut sections as thin as 20 μ quickly and easily. Two of these planes have been used for that purpose for about 7 years.

In the early stages of the investigation, sections of wood and bark were stained by Cartwright's method (11), largely because certain tissue abnormalities were noticed in sections of *P. trifoliata* so stained, and it was thought these might be caused by exocortis virus. Later, phloroglucinol-HCl reagent and safranin-light green stain (14) were used. These are standard stains, and the procedures require no special mention here.

RESULTS.—*Laboratory test for exocortis virus infection.*—Over 1,000 slides of sectioned and stained material were examined microscopically. It seems unnecessary to describe the abnormalities of xylem and bark that were studied without establishing anything worthwhile in relation to exocortis. In the course of these studies it was noticed that a red staining of the phloem tissue was especially heavy in exocortis-affected bark of *P. trifoliata* stained by Cartwright's method. However, staining of the phloem tissues occurred occasionally in sections cut from bark of certain mature trees not affected by exocortis. Later examinations and comparisons indicated that these latter specimens were from sour orange stocks (Satsuma tops, Baldwin County, Alabama). It was discovered that, if attention was limited to the phloem rays and if staining of the other phloem tissue was disregarded, then a very high correlation could be established between the staining reaction and exocortis infection provided the period necessary for incubation had been completed.

Nature of material stained.—It was suspected that the material in the ray cells that stained red with Cartwright's stain was wound gum. Phloroglucinol-HCl, a standard test reagent for wound gum, stained the same areas (see Fig. 1, 2) in the same rays (in virtual serial sections). The procedure of staining with phloroglucinol-HCl was much faster. After application of the reagent and pending examination, the slides were stored in a moist chamber to prevent drying. With phloroglucinol-HCl, color development is complete in 3–4 minutes and remains substantially unchanged for 4 hours.

Microchemical tests.—Sections of fresh, unfixed *P. trifoliata* bark from 2 trees affected with exocortis and from 2 trees free of exocortis symptoms were subjected to a series of microchemical tests. All 4 trees were 40 years old, or more, and probably were infected with other viruses. Tests in which the reagents reacted with exocortis-virus-affected ray cells are presented in Table 1. Affected cells in the rays gave no reaction with the following reagents: zinc-chlor-iodide, for cellulose; Biuret, for peptid-linkage; Millon's reagent, for tyrosine and phenols; and ruthenium red, for pectic materials.

Phloroglucinol-HCl as a reagent for routine tests.—All *P. trifoliata* bark specimens from Louisiana and Florida, except a few damaged in collecting, were retested by the phloroglucinol method. Bark samples were sectioned and processed, and the sections were examined microscopically and diagnosed by serial number. After each lot of 15 specimens had been examined and diagnosed, the results were checked against the field notes for confirmation of diagnosis.

For presentation, the data are segregated into two lots: a) trees with bark scaling, designated as exocortis-positive, and trees of the same age without bark

[5] Through the cooperation of the Florida State Plant Board a small number of citrus trees on *P. trifoliata* rootstock were examined in peninsular Florida. Later, abundant material was examined in Louisiana through the cooperation of Mr. Ralph Brown, Superintendent of Plaquemines Experiment Station, and Mr. M. W. McEachern, Agricultural Agent, Port Sulfur, and through the courtesy of many growers.

scaling symptoms, designated as exocortis-negative (Table 2); and b) trees not scaling but with identity of the parent trees and presence or absence of infection both known (Table 3).

To determine whether unbudded *P. trifoliata* trees presumably free of exocortis virus would react positively to the phloroglucinol test, specimens were examined from 53 seedling trees ranging in age from a

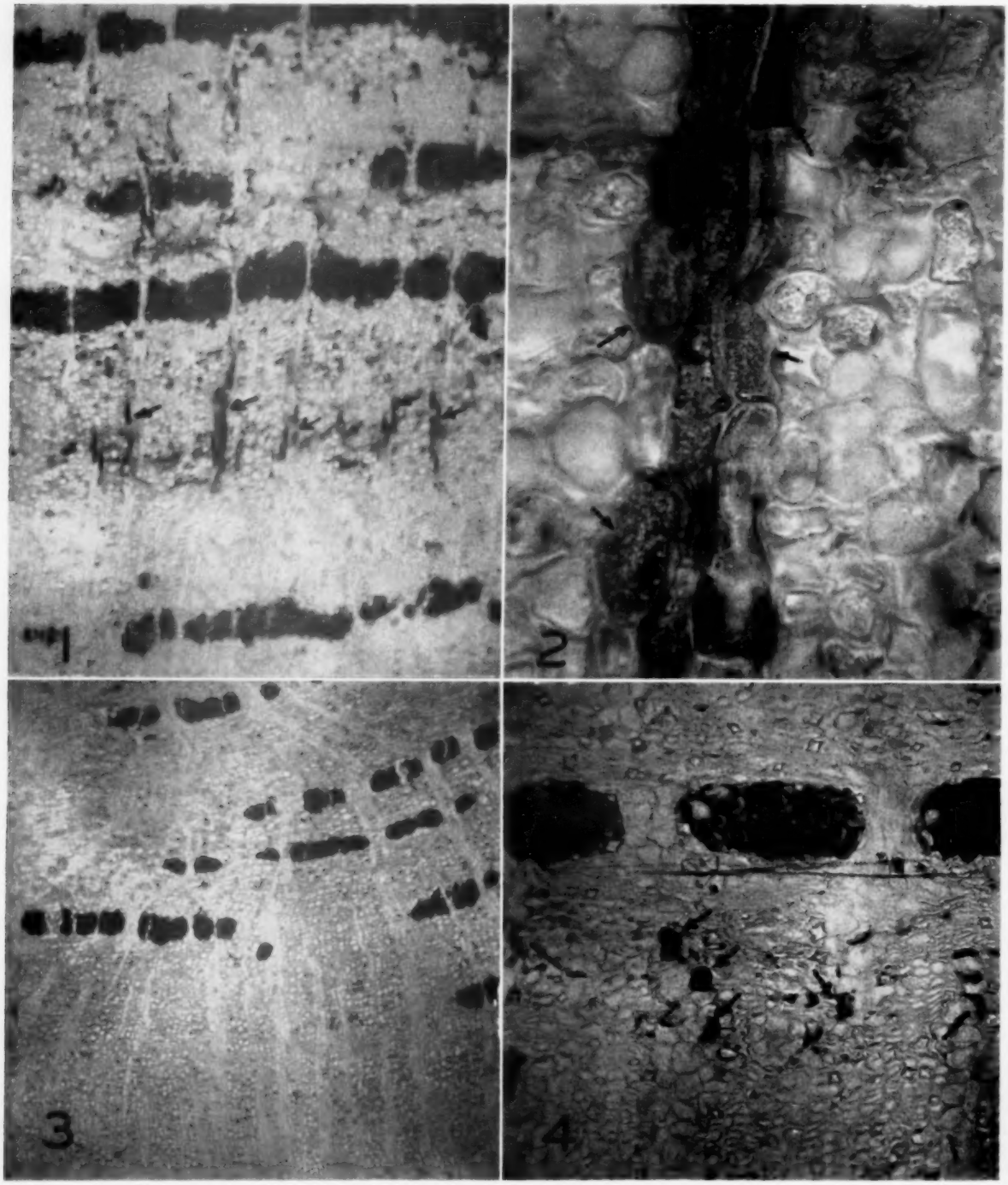

FIG. 1–4. Fig. 1. Cross section of exocortis-infected *P. trifoliata* bark of 40-year-old tree. Affected ray cells stained with safranin-light green. 104×. Fig. 2. Exocortis-affected ray cells in cross section of *P. trifoliata* bark stained with safranin-light green. 970 ×. FIG. 3. Cross section of exocortis-free *P. trifoliata* bark stained with safranin-light green. 62 ×. FIG. 4. Cross-section of *P. trifoliata* bark infected with exocortis but free of psorosis, tristeza, and xyloporosis, stained with safranin-light green. 216 ×.

TABLE 1.—*Microchemical tests of freshly excised, unfixed P. trifoliata bark*[a]

Reagent, test, color	Exocortis-infected trees		Exocortis-free trees	
	Tree 1	Tree 2	Tree 1	Tree 2
Phloroglucinol-HCl a-wound gum—red (brick) b-lignin—red (violet)	red[b]	red[b]	colorless	colorless
Orcinol-HCl a-aldehyde—purple b-pentose—yellow-green	purple[c]	purple[c]	colorless	colorless
Feulgen a-aldehyde—red b-thymonucleic acid—purple	red[b, d]	red[b, d]	colorless[d]	colorless[d]
Schiff's a-aldehyde—red	red[e]	red[e]	colorless[d]	colorless[d]
Ammoniacal $AgNO_3$ a-aldehyde—brown-black	black	black	colorless[d]	colorless[d]
Safranin and light green a-wound gum	red[f]	red[f]	green	green
Mäule a-lignin—red	red[f]	red[f]	colorless	colorless

[a] These tests concern certain cells in the medullary rays of the bark, i.e. those that stain red with phloroglucinol-HCl when the tree is infected with exocortis virus. It is easily possible to distinguish these cells before the reagents are applied by their more opaque cytoplasm. In these tests, cells that show no change from the base color are termed "colorless." Four to 6 sections per slide were treated with each reagent, the test repeated, and the sections re-examined to make sure that the maximum color was reported.

[b] Pomegranate Purple, Ridgway (13). All colors determined at 315 × magnification, with ribbon filament illumination at "high" intensity.

[c] Hyacinth Violet, Ridgway.

[d] Many cells in medullary rays stained Hyacinth Violet. These appeared healthy.

[e] Schoenfeld's Purple, Ridgway.

[f] Rose Red, Ridgway.

few years to 30 or more, in widely separated locations. None of these trees had exocortis-type bark scaling, and all were negative by the phloroglucinol-HCl test (Fig. 3).

Semi-permanent record of the color test.—Because virus-indexing tests conducted under the Florida Citrus Budwood Program might be challenged by a participant, it would be desirable if the slides could be filed after examination, as evidence to support the diagnosis. The transitory nature of the phloroglucinol test makes it worthless as a record, but a semi-permanent record can be obtained by staining some of the bark sections in safranin-light green. This stain provides excellent substantiation of the diagnosis of the phloroglucinol test, and is applied as follows: Transverse sections of bark are cut into a Petri dish containing tap water. Some sections are saved until the phloroglucinol determination has been made. If the diagnosis is positive, a few drops of safranin solution (10 cc of saturated alcoholic solution of safranin in 100 cc of water) are placed in the dish of sections, which is allowed to stand 4–5 hours; overnight is better. When the sections have taken up most of the safranin, several are placed on a slide, blotted dry, and washed momentarily with absolute ethyl alcohol. The sections are then destained in light green (saturated solution in 95 per cent alcohol and clove oil, equal parts), blotted dry, flooded momentarily with xylol, and mounted in Permount or other suitable mounting medium. This schedule is substantially the same as Land's, as described by Chamberlain (5). When properly applied and destained with light green, the safranin duplicates the phloroglucinol-HCl test very nicely (Fig. 1, 2).

Phloroglucinol test for exocortis virus in the presence of other viruses.—From the virus indexing done under the Florida Citrus Budwood Program, it appears that few commercial citrus trees are free of viruses.[6] If that is so, then it may be presumed that many or most of the trees sampled in Florida and Louisiana are infected with viruses other than exocortis. Nevertheless, the correlation of bark scaling to a positive phloroglucinol reaction was very high.

More specifically, most of the trees admitted to the Florida budwood program in 1953 had been indexed on appropriate plants, and virus infections (psorosis, tristeza, and xyloporosis) determined. Thus it was possible to compare the phloroglucinol reaction of trees infected with these 3 viruses in various combinations with the reaction of trees free of them. Over 370 test trees were examined and the staining reaction of the affected cells was just as clear whether or not psorosis, xyloporosis, and tristeza viruses were present (Fig. 4). These data will be presented elsewhere.

Phloroglucinol test applied to several citrus species and 2 hybrids.—Bark specimens were secured from 6 rootstocks of Rangpur lime, 6 of Cleopatra mandarin, and 6 of sweet orange budded 4 years previously with

[6] From unpublished reports of G. G. Norman, who granted permission to cite the information.

TABLE 2.—*Relation between exocortis (bark scaling or scaly butt) of P. trifoliata rootstocks and reaction of ray cells to laboratory test with phloroglucinol-HCl*[a]

Group	Approximate age of trees (years)	Laboratory diagnosis		Field diagnosis		Incorrect diagnosis
		Positive	Negative	Positive	Negative	
A	40	5	–	5	–	0
		–	4	–	4	0
B	25	4	–	4	–	0
		–	7	–	7	0
C	10 to 30	45	–	45	–	0
		1[b]	33	–	34	1
D	15	0	–	0	–	0
		–	8	–	8	0
E	5 to 10	17	2[c]	19	–	2
		–	17	–	17	0
F	8	12	–	12	–	0
		–	2	–	2	0
G	3	0	–	0	–	0
		–	9	–	9	0
H	3	5	–	5	–	0
		–	3	–	3	0
Total				90	84	3[d]

[a] Tops were sweet orange (*C. sinensis*), 129 trees; mandarin orange (*C. reticulata*), 40 trees; grapefruit (*C. paradisi*), 4 trees; (*C. reticulata* × *Fortunella* sp.), 1 tree.
[b] One cell in ray out of 4 sections; probably should have been called negative.
[c] Satsuma trees topworked on sweet orange on *P. trifoliata;* symptoms were weak on all trees.
[d] The 3 errors are probably the result of wrong diagnosis in the field.

Washington navel orange infected with exocortis virus. Bark specimens were also secured from 12 *P. trifoliata* rootstocks, of which 6 were budded 2 years previously with exocortis-infected Washington navel, and 6 were budded at the same time with exocortis-free Washington navel orange.[7] When these bark specimens were subjected to the phloroglucinol test, one Rangpur stock gave a positive reaction. All other 4-year-old trees in the test were negative (Table 4). All 6 of the 2-year-old trees on *P. trifoliata* budded with infected navel orange reacted positively, and all 6 budded with the exocortis-free navel reacted negatively. In addition, mature Rusk citrange-rooted trees, 6 with exocortis scaling and 6 without, were given the phloroglucinol test. The 6 infected trees all gave positive reactions, and the 6 trees without scaling all gave negative reactions.

Distance of phloroglucinol reaction from bud union.—Specimens were taken at various distances from the bud union of 3 exocortis-affected 40-year-old *P. trifoliata* roots with sweet orange tops. The limit of positive reaction to phloroglucinol-HCl was 27 inches linearly below the union in 1 case, and about 18 inches in the other 2. On recently inoculated *P. trifoliata* (13–27 months), the positive reaction was usually limited to tissue not more than ¼ inch (6 mm) from the union. On *P. trifoliata* inoculated 6 months previously, a phloroglucinol reaction was found in cortical parenchyma cells not more than a few cells from the union, but not in ray cells.

[7] Specimens from trees in experimental plots of Ralph Brown, Port Sulfur, Louisiana.

DISCUSSION.—Exocortis virus affects cells in the rays of *P. trifoliata* bark in such a manner that these cells stain Pomegranate Purple (13) when treated with phloroglucinol-HCl, and Schoenfeld's Purple (13) when treated with Schiff's reagent (Table 1). In unstained sections of infected bark, either fixed or fresh, certain ray cells or their contents appear darker (more opaque) by transmitted light than others. The location of affected ray cells ranges from close to the cambium to the region of the crushed phloem in different bark specimens. When many ray cells and numerous rays are affected, the intervening phloem tissue may also stain. Then the outer bark, including most of the phloroglucinol-reacting tissue, frequently sloughs off, giving rise to the bark-scaling characteristic of exocortis (2). On very recently scaled portions of bark, phloroglucinol-HCl may give a negative test. It is suggested that bark scaling is a mechanism by which the plant rids itself of affected tissue. This suggests that species or hybrids of citrus or of *P. trifoliata* that exhibit bark-scaling from exocortis-virus infection can be expected to react positively to the phloroglucinol-HCl reagent. This is tentatively borne out by the positive reaction of Rusk citrange and Rangpur lime. Conversely, it suggests that species or hybrids that do not exhibit bark scaling may not be expected to react in that fashion. The few trees of exocortis-virus-infected sweet orange and Cleopatra mandarin examined (Table 4) bear this out. However, these observations do not resolve the question whether some other virus can or does cause exocortis-like bark scaling in species *other* than *P. trifoliata* or its hy-

TABLE 3.—*Relation between known exocortis virus infection in non-scaling P. trifoliata rootstocks and reaction of ray cells in the bark to laboratory test with phlorogluinol-HCl*[a]

Group	Years from budding	Exocortis virus in bud source tree		Laboratory diagnosis		Incorrect diagnosis
		Positive	Negative	Positive	Negative	
A	4	0		0		0
			25		25	0
B	2	8		7[b]		0
			8		8	0
Total		8	33			0

[a] Tops were Washington navel known to be infected or known to be free of exocortis.
[b] One bark sample damaged in collecting so that sections could not be cut.

brids. If the ray-cell reaction of Rangpur lime is caused by exocortis virus, then it appears to develop more slowly than in *P. trifoliata* (Table 4) (12).

The ray-cell reaction appears to develop first or earliest at the *P. trifoliata* union in contact with the infected top, indicating that bark scaling is a bud-union effect, as pointed out by Benton et al (2). The downward progress of the virus as evidenced by the ray-cell reaction seems to be slow, 18–27 in. in about 40 years (2, 3).

Occasional specimens exhibit a strong reaction to phloroglucinol, primarily in the functional phloem. The cause is not known. At such places there is often an overflow effect, causing adjacent ray cells also to stain. It has been our practice to disregard such rays, making diagnosis on the basis of ray cells adjacent to unstained phloem cells or on the basis of rays in which the staining is much stronger than in adjacent phloem cells.

Histochemical tests indicate that ray cells affected by exocortis virus contain material giving an aldehyde reaction (Table 1). Schiff's reagent without prior hydrolysis gives a plasmal reaction (9) with affected ray cells, indicating the presence of higher aliphatic aldehydes released from acetal phospholipids by the acidic action of the reagent (7).

The presence of cells with darker contents in the rays of *P. trifoliata* bark seems to be a specific effect of the exocortis virus. That such cells contain material that reacts with various aldehyde coupling reagents to give a color reaction seems fortuitous. Other chemical changes could possibly be demonstrated with other reagents.

The question whether viruses other than exocortis may be responsible for the ray-cell reaction in *P. trifoliata* or may mask it seems answered by the correlation between ray-cell reaction and exocortis infection in many orchard trees of all ages, irrespective of top variety and the *presumed* presence of other viruses, and by the fact that ray-cell reaction for exocortis virus was as good in trees also infected with psorosis, xyloporosis, and tristeza as in trees free of them.

Safranin stains wound gum, lignin, pectic compounds, and certain other plant materials. Its combination with light green stain is fortunate in this instance because the latter reduces safranin (8) more readily from unaffected ray cells, permitting a nice differentiation if not allowed to react too long. Fast green was not a satisfactory substitute for light green in this procedure.

The correlation between exocortis virus infection of visibly diseased plants of *P. trifoliata* and a positive reaction of ray cells to phloroglucinol-HCl seems very good considering that only 1 specimen per tree was examined (1.4 per cent error in 215 specimens diagnosed). However the reaction to phloroglucinol is not exhibited uniformly by all rays, and in a given specimen, particularly from a young tree, there may be only a few rays with reactive cells. Thus there is some chance that reactive (affected) ray cells will not be present in a given bark specimen. The reaction becomes more uniform and more pronounced with age of the infection.

TABLE 4.—*Reaction of 3 species of citrus and P. trifoliata to the phloroglucinol-HCl test*[a]

Rootstock	Years from budding	No. inoculated with exocortis	Laboratory diagnosis	
			Positive	Negative
C. aurantifolia	4	6 pos.	1	4[b]
C. reticulata	4	6 pos.	0	5[b]
C. sinensis	4	6 pos.	0	6
P. trifoliata	2	6 pos.	6	0
P. trifoliata (checks)	2	6 neg.	0	6

[a] Bark specimens from trees in a rootstock experiment of Ralph Brown, at Port Sulfur, Louisiana, in which exocortis-infected Washington navel orange was budded across adjacent rows of Rangpur, Cleopatra, sweet orange, and *P. trifoliata* so that buds from a single tree were placed in the first tree of each row. Two years later the *P. trifoliata* seedlings were subbudded from the adjacent sweet-orange-rooted tree. A second series was similarly budded, but with exocortis-free Washington navel orange. The data presented here are taken from the exocortis-infected series (plots 1, 2, 3, and 4) and from the *P. trifoliata* rootstocks (plot 8) of the exocortis-free series.
[b] Bark was so damaged on 1 specimen that it could not be sectioned.

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TWO VIRUSES THAT INDUCE MOSAIC OF APPLE[1]

R. M. Gilmer

SUMMARY

Seven virus isolates that induced symptoms of apple mosaic in apple were compared in host range, modes of transmission, and in a unidirectional cross-protection test. Six of the isolates, 4 derived from apple and 2 from cherry, were similar. They were bud-transmitted to apple and, with 1 exception, to Japanese flowering quince, but not from apple to pear, quince, hawthorn, or *Sorbus aucuparia*. They were not transmitted to any of several herbaceous hosts by expressed sap or by dodder. Differences in rapidity of development of symptoms and amount of foliar chlorosis induced by these isolates appeared to be of a quantitative rather than of a qualitative nature. A single isolate from apple was readily transmitted with expressed sap or with dodder to several herbaceous plant species. It was bud-transmitted to apple, Japanese flowering quince, pear, quince, hawthorn, and *S. aucuparia*. Apple seedlings previously systemically infected with any of the 6 non-sap-transmissible isolates were not protected against infection with this isolate when it was introduced by bud-inoculation. Differences between this isolate and the 6 non-sap-transmissible isolates were regarded as sufficient to justify its description as a new virus.

Yarwood (8) found that an apple mosaic virus (herein referred to as TAMV) could be transmitted with expressed sap or dodder to 13 plant species in 8 families. He did not attempt to identify TAMV with apple mosaic virus (*Marmor mali* H.), noting only that ". . . mechanical inoculations with a similar but different apple mosaic . . . were unsuccessful."

Gilmer (1) and Posnette and Ellenberger (4) independently concluded that line pattern virus (*M. lineopictum* Cation), common in several Prunus species, was identical with apple mosaic virus (*M. mali*). The wide herbaceous host range discovered by Yarwood (8) for TAMV seemed, therefore, to offer several herbaceous plants suitable for detecting latent infections of apple mosaic virus in cherries.

However, numerous attempts in this laboratory to transmit apple mosaic virus to any of these plants from peach, cherry, or apple were invariably unsuccessful. A direct comparison of TAMV with isolates of apple mosaic virus from apple and from cherry was then carried out to determine possible relationships.

Virus sources.—A total of 7 virus isolates, 5 originating in apple and 2 in cherry, were collected.[2] Of the cherry isolates (15-2/1 and A10/1), isolate A10/1 had been shown previously to induce symptoms of apple mosaic in apple (1). Of the 5 apple isolates, 3 originated in New York: apple mosaic virus from a Cortland orchard tree (AMCV), apple mosaic virus from a Red Astrachan nursery tree (AMSV), and 51A1 from a Hubbardston orchard tree. Isolate TAMV, previously characterized fully by Yarwood (8), originated in an unknown apple variety in Tulare County, California; it differs in origin and behavior from the apple mosaic virus described by Thomas (6).[3] Isolate AP-4, obtained from a Jonathan tree near Prosser, Washington, had been shown by Kirkpatrick (2) to be

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[2] Drs. C. E. Yarwood, E. C. Blodgett, K. G. Parker, and D. H. Palmiter kindly supplied certain of the apple mosaic isolates.

[3] Letter from Dr. H. Earl Thomas, December 4, 1957.

TABLE 1.—*Rapidity of symptom development and symptom severity in apple of virus isolates*

Isolate	Days from bud-inoculation to appearance of symptoms		Symptom intensity[a]
	85°F	70-50°F	70-50°F
TAMV	7–10	15	very intense
AP-4	6–13	13–19	very intense
51A1	9	15	intense
AMCV	11–14	17	conspicuous
AMSV	28–35	26–33	conspicuous
A10/1	23–30	32–37	slight
15-2/1	21–30	22–37	slight

[a] Intensity classified after Posnette and Cropley (3), but little or no necrosis or defoliation occurred in the "very intense" and "intense" classifications.

transmissible to peach, where it induced symptoms of line pattern.

All of the isolates were transmitted by bud-inoculation to apple seedlings, which were used as sources of inoculum.

COMPARISON OF SYMPTOMS IN APPLE.—Each isolate was bud-inoculated in February, 1956, to a separate group of 4 apple seedlings just as they were breaking dormancy in the greenhouse. Since temperature had been shown to influence the amount of leaf chlorosis in apple seedlings infected with apple mosaic virus (1), half of the trees of each inoculation series were grown at 85°F, and the remaining half at 70°F (days) and 50°F (nights).

Both temperature and source of inoculum influenced rate of development and amount of foliar chlorosis (Table 1). Irrespective of inoculum source, chlorosis in trees of the inoculation series was more pronounced in those grown at 70–50°F than in those grown at 85°F. At 70–50°F, the chlorotic areas were yellow at first and remained so for an extended period; at 85°F, the initial yellow coloration quickly bleached to white.

Leaf and bud necrosis in trees of the TAMV, AP-4, and 51A1 inoculation series occurred in those grown at 70–50°F, but not in those grown at 85°F. The same isolates induced more pronounced chlorosis than did any of the other isolates in trees grown at either temperature.

As greenhouse temperatures increased, in late June and July, the amount of foliar chlorosis developing in all of the inoculation series progressively decreased. Symptoms of infection on new leaves became almost completely masked except in trees inoculated with TAMV. Trees infected with this isolate continued to develop a small amount of chlorosis, which was confined to the tips of the leaf serrations.

TRANSMISSION BY BUD-INOCULATION TO OTHER ROSACEOUS PLANTS.—Each isolate was bud-inoculated from apple to 3 plants of each of the following species: Japanese flowering quince (*Chaenomeles lagenaria* (Loisel) Koidz.), quince (*Cydonia oblonga* Mill.), pear (*Pyrus communis* L.), hawthorn (*Crataegus chlorosarca* Maxim.), and *Sorbus aucuparia* L.

The data are summarized in Table 2. Virus was not recovered by bud-inoculation to apple from inoculated plants that did not develop symptoms, indicating that such plants were not symptomless virus carriers.

TRANSMISSION BY SAP-INOCULATION FROM APPLE FOLIAGE.—Attempts were made to transmit each isolate by inoculation of various herbaceous plants with sap extracts from infected apple leaves. About 0.5 g of young leaf tissue from systemically infected trees was triturated in 2 ml of diluent, and the resultant extract was gently rubbed with a cheesecloth pad on the foliage of test plants previously dusted with 400-mesh carborundum. Various diluents were used, including distilled water, .01 M phosphate buffers of pH 5.5, 6.5, 7.5, and 8.5, and sodium bisulfite at .01 and .001 M.

TAMV was the only isolate transmitted in any of these experiments. It infected seedlings of cucumber, tobacco, bean (var. Pencil Pod), *Tithonia speciosa* L., and *Nicotiana glutinosa* L. When young leaf tissue was used, inoculum prepared with distilled water was consistently infective. Older leaves with marked symptoms provided inoculum of little or no infectivity.

In 2 experiments in which the fresh leaf-disk inoculation method of Yarwood (7) was used, TAMV was transmitted to tobacco and cucumber; none of the remaining 6 isolates were transmitted to either.

TRANSMISSION BY DODDER.—Virus-free dodder (*Cuscuta subinclusa* D. & H.) grown from seeds was established on apple seedlings infected with each of the virus isolates. Following establishment, dodder strands were trained on young tobacco and apple seedlings.

Severe necrosis developed in 11–21 days in all of 6 tobacco seedlings on which dodder was trained from a tree infected with TAMV. TAMV was also dodder-transmitted to 11 of 12 apple seedlings. TAMV was readily transmitted by sap from triturated dodder to tobacco seedlings, and also, as previously reported by Yarwood (8), was recovered from sap-inoculated tobacco plants to apple by means of dodder.

None of the other 6 isolates was dodder-transmitted to tobacco or apple seedlings, nor was any of them mechanically inoculable to tobacco, *Tithonia*, or cucumber seedlings with sap from triturated dodder.

CROSS-PROTECTION.—Each of 6 series of 4 apple seedlings was bud-inoculated in April, 1956, with one

TABLE 2.—*Infectivity of virus isolates bud-inoculated from apple to other Rosaceae*

Species	Isolate						
	TAMV	AP-4	51A1	AMCV	AMSV	A10/1	15-2/1
Chaenomeles	+[a]	+	+	+	+	+	–
Sorbus	+	–	–	–	–	–	–
Crataegus	+	–	–	–	–	–	–
Pyrus	+	–	–	–	–	–	–
Cydonia	+	–	–	–	–	–	–

[a] +indicates that symptoms developed and virus was recovered after inoculation; –indicates that symptoms did not develop and virus was not recovered after inoculation.

of the non-sap-transmissible isolates (AMCV, AMSV, 51A1, AP-4, 15-2/1, and A10/1). The following August, at least 3 months after the seedlings had developed symptoms, 2 trees in each inoculation series were additionally bud-inoculated with TAMV; the other 2 trees were retained as controls.

In February, 1957, after a dormant period, the apple seedlings were forced in the greenhouse and assayed for TAMV by sap inoculations to tobacco seedlings.

TAMV was recovered from all of the trees to which it had been inoculated. No virus transmission to tobacco was obtained from the control trees that had not been additionally inoculated with TAMV.

Discussion.—Posnette and Cropley (3) have shown that mild strains of apple mosaic virus afforded a high degree of protection against the effects of more virulent strains introduced by grafting. Because they were unable to transmit mechanically any of the numerous apple mosaic strains with which they worked, they predicted that TAMV would be found distinct from apple mosaic virus.

The present data show that TAMV, although it induces symptoms in apple that cannot be distinguished from those caused by apple mosaic virus, is not closely related to apple mosaic virus. TAMV and apple mosaic virus differ greatly in host range and modes of transmission. Complete cross-protection tests between TAMV and apple mosaic were not possible, because of the inability to detect apple mosaic when TAMV was present. In a unidirectional cross-protection test, any of 6 isolates of apple mosaic virus failed to immunize infected apple seedlings against superimposed infection with TAMV.

Since some confusion of the 2 viruses is already present in the literature (cf. Smith (5), pp 19-23), it appears advisable to characterize TAMV as a new virus:

Marmor decipiens, n. sp. The specific epithet is chosen because of the similarity of symptoms induced in apple to those induced by apple mosaic virus (*M. mali* H.).

Common name: Tulare apple mosaic virus.

Geographical distribution: Tulare County, California.

Induced diseases: In apple, *Helianthus annuus* L., and *Tithonia speciosa* L., a chlorotic mosaic of foliage. In some varieties of *Phaseolus vulgaris* L., necrotic local lesions; in *Nicotiana tabacum* L., local etched rings, systemic necrotic shock, recovery with virus present.

Host range: An extensive host range is found in Yarwood (8); additionally, *Crataegus chlorosarca* Maxim., *Sorbus aucuparia* L., *Cydonia oblonga* Mill., *Pyrus communis* L., and *Chaenomeles lagenaria* (Loisel) Koidz.

Transmission: By grafting, by dodder, by expressed sap.

Relationships: Suggested relationship or identity with tobacco streak virus (8) not established.

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VIRUS GUMMOSIS OF APRICOT [1]

Earle C. Blodgett and James A. Twomey [2]

SUMMARY

A new disease of *Prunus armeniaca* L., affecting Royal and Tilton and caused by a virus easily transmitted by budding, is named virus gummosis. Symptoms consist of profuse gumming on branches and trunks, necrosis and dieback of new shoots, a lace-leaf effect, and dead, punky areas in the fruit.

In 1947 a fruit grower near Granger, in the Yakima Valley of Washington, called our attention to an apparently new disease affecting apricots, *Prunus armeniaca* L. Since that time 3 other orchards (2 in 1955) have been found with the same disease. Its potential seriousness and apparent differences from known apricot disorders [3] make appropriate a record of symptoms, distribution, and transmission data.

SYMPTOMS.—Virus gummosis occurs on Royal (Blenheim) and Tilton apricot varieties but does not appear to naturally affect Wenatchee [4] in the same orchard. The disease has appeared in well over half the Royal trees in the original orchard, making their removal necessary. The most striking symptom is the gum produced in enormous quantity on affected branches and trunks (Fig. 1,D). The smaller branches develop necrotic areas, the bark becomes roughened and discolored, and gumming on these areas is also common. Lesions formed on the current year's shoots eventually kill the twigs and buds. Characteristically affected trees die back extensively and, the following year, push out fewer, but vigorous, water sprouts. These may later die back nearly or completely to the main branch or trunk. This dieback is repeated 3 or 4 years, until the tree is dead. Most affected trees are so seriously impaired that they are removed after a season or two.

Fruit sets sparingly and some may be normal in the early stages of tree infection. Fruit that does set and show symptoms usually falls early, and, by harvest time, practically no fruit can be found on severely affected trees or branches. At first examination, virus-gummosis-diseased fruit (Fig. 1,B-C) shows some similarity to fruit affected by apricot ring pox.[5] The former, however, are more misshapen, there is more necrosis of tissue deeper in the fruit, and conspicuous rings and spots are absent. Necrotic spots and areas form on the leaves, later falling out and giving the appearance of lace leaf. Many of the leaves turn yellow and drop before fruit maturity. The current year's growth of spurs and shoots, if severely affected, may wither and blast, as though scorched by fire (Fig. 1,B).

TRANSMISSION TESTS.—In a preliminary experiment, budwood was collected from affected Royal apricot trees in the orchard where the disease was originally reported. In 1948, buds were placed on Lovell peach seedlings in plots at the Irrigation Experiment Station, Prosser, Washington. Some seedlings received buds only from diseased trees, some only from healthy trees; other seedlings received both, a healthy bud of Royal above the diseased inoculating bud in some cases and below it in others. Shoots grew from most of the buds, but nearly all of the young trees that had been inoculated, including the understock, eventually died. The shoots from healthy buds on double-worked trees grew the next season, but during the succeeding winter and summer, succumbed, with profuse gumming. Trees with only healthy buds grew normally. These tests indicated that a virus might be involved.

In another experiment, budwood of the current year's shoots in the early stages of necrosis and gumming was collected from diseased trees in the second diseased orchard that had been located. On July 18, 1951, 4 buds were set on each of the main scaffold branches of 5 2-year-old Tilton apricot trees in the plots at Prosser. Alternate trees in the row were inoculated, leaving an uninoculated check between each inoculated pair (Fig. 1,A). During late fall, and early the next season after growth started, the tissue near the point of bud insertion died and gummed profusely. The affected area extended up and down the branches and, later in the same year, appeared on other branches and on the main trunk. One tree died during the first year after inoculation, and 1 more during the second. The remaining 3 trees survived until the spring of 1955, when all died. The trees had fruited well, and there were no clear-cut fruit symptoms. Fruiting in the Royal variety may be severely affected by virus gummosis although the ring pox virus produces no symptoms on this variety.

In 1952 and 1953, buds were taken from 3 of these inoculated trees and used to inoculate additional trees in the same row, about 100 feet distant. Sequence of symptom development was similar to that in the previ-

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The authors gratefully acknowledge constructive criticism of the manuscript by Dr. L. C. Cochran.

[3] Bodine, E. W., and E. L. Reeves. 1951. Ring pox of apricot, p. 187-188, in Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Agr. Handbook No. 10. 276 p.

[4] The variety herein called Wenatchee has formerly been widely known as Wenatchee Moorpark. Since there is no logical reason for connecting the Moorpark with the newer variety, many leading horticulturists feel the name Wenatchee is correct, and it is now in fairly general use.

[5] Cochran, L. C., and G. L. Stout. 1953. Apricot ring pox. Calif. Dept. Agr. 42: 7-10.

FIG. 1. Virus gummosis of apricot. A) Nine Tilton apricot trees, 5 inoculated with affected budwood and 4 check trees. B) Naturally infected Royal apricot. Note lesions on twigs, fruit and leaves, and blasting of spurs. C) Royal apricot fruit showing lesions and gumming; natural infection. D) Tree 1 in A showing gummosis.

ous test. Uninoculated trees have remained normal.

It was realized that some microorganisms might have been transferred to the inoculated plants and were involved in producing gummosis. Isolation attempts, while not exhaustive, failed to reveal a parasitic organism.

In 1954, 2 trees each of several *Prunus* varieties were inoculated by budding from diseased trees. During the next 2 years, no symptoms were detected on Montmorency cherry, *Prunus cerasus* L.; Italian prune, *P. domestica* L.; Napoleon (Royal Ann) cherry, *P. avium* L.; or on Christensen, T-101 (a numbered early-maturing selection), and Newcastle Early apricots. There was profuse gumming and necrosis locally around inoculating buds on Chinese [6] apricot, and much more extensive injury on Henderson and Wenatchee apricots. Winter injury made accurate reading impossible on Brooks and Wilson apricot. The one check tree in each series did not show symptoms.

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INHERITANCE OF RESISTANCE TO SOUTHERN ANTHRACNOSE IN RED CLOVER [1]

Kirk L. Athow and Ralph L. Davis [2]

SUMMARY

The F_2 segregation in a cross of a resistant and a susceptible red clover selection was studied under artificial infection with *Colletotrichum trifolii*, the causal fungus of southern anthracnose. The F_2 progenies from each of 14 F_1 plants as well as the total population segregated in a ratio of 3 susceptible to 1 resistant, indicating that resistance is controlled by a single, recessive Mendelian factor.

Southern and northern anthracnose of red clover caused, respectively, by *Colletotrichum trifolii* Bain & Essary and *Kabatiella caulivora* (Kirchn.) Karak., are recognized as destructive diseases in certain areas of the United States. Mainly because of temperature requirements, southern anthracnose is most prevalent in the southern part of the clover belt, and northern anthracnose is more important northward. Both diseases frequently occur in Indiana and other midwestern states.

The first report of southern anthracnose was by Bain and Essary (4), on red clover in Tennessee in 1905. The following year they described the causal organism as *Colletotrichum trifolii* (5). A number of workers have noted differences in the susceptibility of red clover strains to the fungus (1, 2, 6, 7, 8, 9, 10, 11). It was the general consensus that resistant strains were the only practical means of control. All attempts to develop resistant strains have been through selection. The Kenland variety developed in this way has good field resistance to southern anthracnose, but is very susceptible to northern anthracnose.

One phase of the clover improvement program at Purdue, begun in 1951, was an attempt to combine, in a single strain through hybridization, the southern anthracnose resistance of the variety Kenland with the northern anthracnose resistance of the varieties Dollard or Purdue. Southern-anthracnose-resistant Kenland plants and northern-anthracnose-resistant Dollard and Purdue plants were selected as parental material. Nearly 5000 F_2 progenies from Kenland $\times$ Purdue and Kenland $\times$ Dollard crosses were inoculated with *K. caulivora*. About 75 per cent of the plants, proving susceptible, were discarded. The remaining plants were inoculated with *C. trifolii*. Again, about 75 per cent of the plants were susceptible. Although the reaction of each parent was known to only 1 disease and a composite of several isolates of each organism was used, the proportion of susceptible to resistant plants suggested that resistance to each disease might be controlled by an independent, single, recessive factor. Recognizing that the most effective breeding program was contingent on understanding the mode of inheritance, independent inheritance studies were initiated with northern and southern anthracnose. A brief account of some of the work has already appeared (3).

MATERIALS AND METHODS.—Fourteen F_1 plants were obtained from crosses between Res. 7-1 (a southern-anthracnose-resistant selection) and Sus. 5 (a southern-anthracnose-susceptible selection). The F_1 plants were caged together in the greenhouse and interpollinated by bees. The F_2 seedlings were inoculated at 38 days with a spore suspension of single-spore isolate 21-2 of *C. trifolii*. The infected plants were counted and removed in about 10 days. Remaining plants were reinoculated 3 times at 20-day intervals. Following the third inoculation, only 1 plant of the susceptible check was not infected. The variety Dollard was used as the susceptible check.

EXPERIMENTAL RESULTS.—The F_2 progenies from each of the 14 F_1 plants segregated in a ratio of 3 susceptible to 1 resistant (Table 1). The total population also segregated into a satisfactory fit to a 3:1 ratio, indicating that resistance was controlled by a single, recessive Mendelian factor. Only 1 of 125 plants of the variety Dollard remained disease-free.

There were some differences in degree of suscepti-

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[6] Represents stock of Chinese, from Utah; probably also known as Large Early Montgamet. Not of Chinese origin.

TABLE 1. *Segregation for reaction to Colletotrichum trifolii in the F_2 populations from 14 F_1 red clover plants.*

F_1 plant number	Number of F_2 plants			Chi-square test of 3:1 ratio[a]
	Total	Susceptible	Resistant	
5	63	41	22	3.3
6	18	12	6	0.7
7	30	25	5	1.1
8	39	27	12	0.7
9	41	32	9	0.2
10	9	6	3	0.3
12	33	28	5	1.7
13	19	14	5	0.0
14	15	10	5	0.6
15	4	3	1	0.0
18	8	5	3	0.7
19	17	13	4	0.0
20	8	7	1	0.7
21	18	13	5	0.1
Total	322	236	86	0.6

[a] Chi-square values smaller than 3.8 indicate a satisfactory fit to the expected ratio at the 5% level.

bility among the susceptible segregates, and marked differences between some of them and the susceptible variety Dollard. Many entire plants of the Dollard variety were killed, while few of the susceptible segregates died. For the most part, infection on the susceptible segregates was restricted to a few stems, petioles, petiolules, or leaflets. This type of reaction is found in a high percentage of plants when lots of Kenland are artificially inoculated, although an attempt has been made to maintain its resistance by continual reselection within the variety. This susceptibility is not surprising in view of the fact that resistance is recessive. A few dominant, susceptible escapes could increase the percentage of susceptible plants in subsequent generations since red clover is almost entirely cross-pollinated. Preliminary studies indicate the possible existence of races of *C. trifolii* differing in pathogenicity. This could also account for the difficulty in maintaining complete resistance in the variety Kenland.

PURDUE AGRICULTURAL EXPERIMENT STATION
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PROPERTIES, ASSAY, AND PURIFICATION OF WHEAT STREAK MOSAIC VIRUS [1]

Myron K. Brakke [2]

SUMMARY

Wheat streak mosaic virus was partially purified by density-gradient centrifugation and zone electrophoresis. The addition of an extract from healthy wheat increased the infectivity titer and decreased the stability of the purified virus. One per cent added sucrose had no effect on its stability. Infectivity assays were performed by determining the percentage of infected plants inoculated with various dilutions. The loglog of the inverse of the fraction of plants remaining healthy was plotted against the logarithm of the concentration to give a straight line with a slope of 1.

INTRODUCTION.—Wheat streak mosaic virus, which causes a common disease of wheat in the Great Plains area, is transmitted by the mite *Aceria tulipae* (Keifer) (14). Many papers have appeared on various aspects of the disease caused by this virus, but few on the properties of the virus itself. McKinney (10) stated that the virus was inactivated by heating infectious sap 10 minutes at 55°C. Gold, Houston, and Oswald (9), who reported that sinuous rods 15 × 670 mμ were associated with the disease, were unable to obtain a highly purified virus because of its instability. Burger (7) reported that the virus was more unstable when partly purified.

This paper describes experiments on purification with density-gradient centrifugation and zone electrophoresis used in conjunction with procedures for partly denaturing normal proteins. The stability of the virus to certain of these denaturing procedures was also investigated. Data will be reported elsewhere on correlation of the sinuous rods seen in the electron microscope with the infectivity of fractions from some of the steps in the purification procedure.

MATERIALS AND METHODS.—*Virus source.*—The type strain of wheat streak mosaic virus ATCC No. 29 (1) was obtained from H. H. McKinney. The virus was transmitted by *A. tulipae*[3] to wheat plants that were used as the primary source of inoculum for stock cultures. Either Cheyenne or Michigan Amber winter wheat was used as host for the virus for both assay and source. Wheat plants were inoculated in the two-leaf stage by leaf stripping with a cheesecloth pad dipped in inoculum containing suspended 500-mesh corundum or diatomaceous silica (Celite, manufactured by Johns Manville Co.). Plants were grown under ordinary greenhouse conditions at 70–100°F. An evaporative cooler cooled the greenhouse in summer. Small amounts of diseased leaf tissue were ground in a mortar; large amounts were put through a meat grinder. All grinding and subsequent purification of extracts were done in a room kept at 2 ± 1°C. Unless specified otherwise, a Spinco Model L preparative ultracentrifuge was used for all centrifugation.

Density-gradient centrifugation.—Gradient columns for rate zonal centrifugation were made by layering 4, 7, 7, and 7 ml of solutions containing, respectively, 100, 200, 300, and 400 g sucrose per liter in 1 × 3-in. nitrocellulose centrifuge tubes at least 1 day before they were to be used (4, 6). Unless otherwise stated, the sucrose was dissolved in a buffer of pH 7.2 that had 0.0067M K_2HPO_4 and 0.0033M KH_2PO_4. After a layer of virus solution (2–5 ml) had been floated on each of the gradient columns, they were centrifuged at 24,000–25,000 rpm (mean R.C.F. 64,000 × G) in the SW-25.1 rotor. Temperature of the tube contents after centrifugation was usually 5°C. Samples were usually removed from the centrifuged tubes into a syringe through a 4-in. 20-gauge needle with a bent tip, which was slowly inserted into the column from the top. Samples were sometimes removed by puncturing the sides of the tube with a 1-in. 22-gauge needle.

Gradient columns for equilibrium zonal centrifugation were made by layering 4 ml each of solutions containing 300, 400, 500, and 600 g sucrose per liter in a 1 × 3-in. centrifuge tube or by layering 0.9 ml of each of these solutions in a ½ × 2-in. centrifuge tube. One ml of virus preparation could be floated on the sucrose solution in the ½ × 2-in. tubes, which were then centrifuged 3–3½ hours at 36,000 rpm (mean R.C.F. 100,000 × G) in the SW-39 rotor. Five ml of virus preparation from the rate zonal centrifugation tubes was floated on each of the equilibrium gradient columns in the 1 × 3-in. tubes. Then 7 ml of water was floated on top of the virus layer to prevent collapse of the tubes. Finally, the tubes were centrifuged 7 hours at 24,000 rpm in the SW-25.1 rotor.

Zone electrophoresis.—The gradient columns contained a sucrose concentration ranging either from 300–600 g/liter or 0–400 g/liter over a depth of 8 cm (5). The former were used for virus preparations from equilibrium zonal centrifugation, and the latter for those from rate zonal centrifugation. The buffer most commonly used had 0.01M KCl, 0.005M K_2HPO_4, and 0.0005M KH_2PO_4 with pH of 7.7–7.8. Sufficient voltage was applied to give a power release of 2–3 watts per U-tube. Electrophoresis was allowed to proceed until the virus had migrated 2–3 cm.

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[3] The mite transmission was performed by Dr. R. Staples, Department of Entomology, University of Nebraska.

Infectivity assay.—A series of dilutions, at either whole or half log units of the solution to be assayed, was prepared in 0.01M neutral potassium phosphate buffer containing 1 per cent Celite. All dilutions were based on the original weight of plant tissue from which the extract was made. Each dilution was used to inoculate 30–50 wheat plants, in three 4-in. pots. As controls, the plants in 3 pots were inoculated with buffer used for the dilutions. If any of these showed symptoms, the experiment was discarded. The number of systemically infected plants was counted 10–14 days later. Preliminary estimates of virus concentration were made by plotting loglog $\frac{(\quad 100 \quad)}{(\%\text{ healthy plants})}$ against log dilutions, drawing the straight line with a slope of minus one that appeared to give the best fit through the points, and estimating the dilution that would have infected 63 per cent of the plants. Values of concentrations and the standard deviations reported in the tables were calculated by the maximum likelihood method of Finney (8).

In all stability experiments a few infected assay plants inoculated with concentrations near the dilution end point were analyzed by rate zonal centrifugation for wheat streak mosaic virus. In all cases a typical zone was obtained that sedimented 85–90 per cent as fast as tobacco mosaic virus, indicating that the virus measured in these experiments was actually wheat streak mosaic virus instead of some contaminant picked up during the course of the work. Every 6 months the stock cultures were renewed from dried leaves of plants inoculated by mites. This should be a reliable procedure for eliminating other viruses since this is the only mechanically transmissible virus of wheat known to be transmitted by this mite.

Results.—*Accuracy of assay.*—The Poisson equation was first applied to interpreting infection by plant viruses by Youden, Beale, and Guthrie (16). Application of the equation in the present manner was first reported by Plus (12), for interpreting the assay of the sigma virus of *Drosophila melanogaster*. Briefly, the main assumptions are that all susceptible sites have the same susceptibility, all inoculated plants have the same number of susceptible sites, and 1 virus particle may initiate an infection if it is sufficiently close to the susceptible sites, i.e. in the "infection initiating volume." The probability that an assay plant will remain healthy is equal to the probability (P_o) of finding no infectious particles in the "infection initiating volume." According to the Poisson equation, $P_o = \frac{s^o e^{-s}}{O!} = e^{-s}$, where s is the average number of particles in the infection-initiating volume, and e is the base of natural logarithms. Since s is directly proportional to the concentration, $s = kc$. The probability that a plant will remain healthy is approximated by p, the fraction of assay plants that remains healthy. The above equation then reduces to $\log_e p = -kc$, where k is a constant and c is the concentration of virus. Since it is more convenient to use the logarithm of the concentration, this equation may be changed to $\log_e \log_e \frac{1}{p} = \log_e k + \log_e c$. A plot of $\log_e \log_e \frac{1}{p}$ against $\log_e c$ should give a straight line with a slope of 1. In a dilution series, c is the "dilution," or reciprocal of the dilution factor (i.e. $c = 1/10$ for a 10-fold dilution), and k is then the concentration of virus particles per infection-initiating volume in the original solution (1:1 dilution). When there is an average of 1 infectious virus particle per infection-initiating volume, $s = 1$, $P_o = 0.37$, and $\log c = -\log k$. Therefore -1 times the logarithm of the dilution that results in infection of 63 per cent of the plants equals the logarithm of the concentration of infectious virus particles per infection-initiating volume in the original solution. Results in this paper are expressed as logarithm to the base 10 of the number of infectious particles per infection-initiating volume.

There are a number of sources of error in this method that cannot be evaluated on a theoretical basis. Among these are variation in the susceptibility of the infection sites, variation in the number of susceptible sites, and the effect of "environmental factors." For purposes of discussion, all of these may be put together as variation in size of the infection-initiating volume. There is also an error in setting P_o equal to p. Variance of this estimate should be the usual proportionality variance, $\frac{P_o(1-P_o)}{N}$ where N is the number of plants inoculated at 1 dilution. The corresponding variance in $\log_e \log_e \frac{1}{p}$ is $\frac{1-P_o}{P_o N (\log_e P_o)^2}$ (12). The variance in the concentration calculated from the formula $\text{loglog}\frac{1}{p} = \log k + \log c$ will be at least this large.

Three separate assays were made of the same preparation to determine experimentally the magnitude of the over-all error of the assays (Table 1). The original preparation was a crude extract from infected wheat plants that had been clarified by low-speed centrifugation. Three separate dilution series were made of this preparation: $10^{-1.0}$, $10^{-1.5}$, $10^{-2.0}$, $10^{-2.5}$, $10^{-3.0}$, and $10^{-3.5}$ for assay "A"; $10^{-1.3}$, $10^{-1.8}$, $10^{-2.3}$, $10^{-2.8}$, $10^{-3.3}$, and $10^{-3.8}$ for assay "B"; and $10^{-1.1}$, $10^{-1.6}$, $10^{-2.1}$, $10^{-2.6}$, $10^{-3.1}$, and $10^{-3.6}$ for assay "C." In each experiment, all results from the 3 assays were combined, each point weighted with the inverse of its variance, and the slope of the best-fitting straight line calculated by the method of least squares using formulae given by Bliss (3). The weight of each point was calculated from the expected value of p at that dilution, which was determined graphically from a preliminary plot of the data. The slopes and their

standard deviations are given in column 2 of Table 1. In 2 experiments a slope of about 1 was obtained; in the other 2 it was less than 1. The chi square (χ^2) test shows that the data fit these lines satisfactorily except in the last experiment, where chi square is just significant at the 5% level (3).

The slopes in Table 1 were calculated after combining points from 3 assays. Standard deviations of slopes calculated from points of a single assay would be even larger than those given in Table 1. Instead of calculating a slope for each assay or each group of assays, it has been assumed that all assays would fit a line of the same slope. A slope of 1 has been used even though it appears from data in Table 1 that a slope of somewhat less might give the best fit to the most assays. A slope of 1 simplifies calculations, and, since there is a theoretical basis for it, it is also of interest to know whether a line of this slope fits the data.

Finney (8) has given a maximum-likelihood iterative procedure using this loglog transformation for calculating the concentration of bacteria in a culture. His method of calculation was used for the present assays, with results in Table 1. Experiments with an empirical slope significantly different from 1 also gave a significantly high value of chi square with this method of calculation. In these cases the variance of the estimate of log k was multiplied by $\frac{\chi^2}{\text{d.f.}}$ where d.f. is the degrees of freedom (3). Following Finney's procedure, it may be assumed that the errors in log k follow a normal distribution, and the 95 per cent confidence limits are given by 1.96 times the standard deviation. Since the 95 per cent confidence intervals of all the replicate assays in Table 1 overlap, it is concluded that this method of calculating the concentration and its standard deviation satisfactorily describes the error in this assay.

This maximum-likelihood iterative method usually requires only 1 cycle of calculation, but nevertheless is quite time-consuming. A simpler procedure is to determine graphically the dilution that would infect 63 per cent of the assay plants, by plotting $\text{loglog}\frac{1}{p}$ against log c. The results of this procedure for the assays reported in Table 1 are given in column 6 of that table. This is equivalent to an unweighted average since the line through each set of points was drawn so that the absolute values of the positive and negative errors were equal. The same results would have been obtained by calculating log k from each assay and averaging the results. This procedure allows one to judge roughly how well the assay conforms with the theory, but it does not indicate the error of the estimate of concentration.

Correcting the standard deviation in assays with a high value of chi square by multiplying the variance by $\frac{\chi^2}{\text{d.f.}}$ is a procedure that must be used with discretion. Varying the size of the infection-initiating volume, especially when p is either very high or very low, can result in a high value of chi square. If only 1 or 2 plants remain healthy after inoculation with a high concentration of virus, or become diseased after inoculation with a low concentration, a high value of chi square will often result. In such cases, the above correction should be justified. In other cases, a high value of chi square may be consistently associated with some treatment of the virus, and may indicate aggregation of the virus, the presence of substances affecting virus stability, or the presence of substances affecting the infection process. In these cases, the dilution curve obtained is probably not a result of virus concentration alone. Virus concentrations from such dilution curves should be compared with caution, regardless of method of calculation.

Calculation of virus concentration from the fraction of plants that remain uninfected at only 1 dilution

TABLE 1.—*Accuracy of infectivity assay for wheat streak mosaic virus*

Expt. No.	Slope	χ^2 for slope	d.f.[a]	Replicate	Log_{10} k[b] Unweighted average	Log_{10} k[b] Maximum likelihood	χ^2	d.f.[a]
1	0.53 ± 0.21	3.27	6	A	3.35	3.20 ± 0.33[c]	47	2
				B	3.35	3.32 ± 0.17	12	2
				C	3.35	3.34 ± 0.07	2.8	1
2	1.03 ± 0.16	13.0	12	A	1.90	2.00 ± 0.06	4.8	4
				B	2.10	2.11 ± 0.07	4.8	4
				C	1.95	2.09 ± 0.06	4.1	3
3	0.64 ± 0.14	14.0	13	A	3.35	3.39 ± 0.22	46.7	3
				B	3.05	2.88 ± 0.06	10.9	5
				C	3.30	3.12 ± 0.20	51.7	4
4	0.96 ± 0.22	22.7	12	A	2.30	2.24 ± 0.07	9.5	4
				B	2.45	2.35 ± 0.06	7.2	4
				C	2.10	2.13 ± 0.07	2.5	3

[a] Degrees of freedom.
[b] This is the logarithm of the number of infectious particles per infection-initiating volume, and is equal to −1 times the logarithm of the dilution that infects 63% of the assay plants.
[c] Standard deviation. The 95% confidence limits are given by 1.96 times the standard deviation.

must be considered to have a larger error than that due to the proportionality variance alone, particularly if p is very high or very low. There is no way of testing a 1-point assay for goodness of fit or significant value of chi square. Furthermore, the frequency distribution of $\log\log\frac{1}{p}$ is slightly skewed at low and high values of p.

As with all bioassays for plant viruses, conclusions as to relative concentrations can be made only for those assays performed at the same time.

Effect of denaturing procedures.—Preliminary experiments showed that the infectious entity sedimented 85–90 per cent as fast as tobacco mosaic virus in rate zonal centrifugation. However, no zone was seen with an untreated crude extract. Freezing a crude extract did not result in a visible zone. However, if a crude extract prepared by grinding infected wheat leaves with twice their weight of water was adjusted to pH 6.0–6.5, heated an hour at 40°C, clarified by low-speed centrifugation, and then given rate zonal centrifugation (2 hours), a zone was visible at a depth of about 2.0–2.5 cm. If 2 ml of clarified extract was floated on the gradient column, the resulting zone was usually 2 mm wide, with sharp edges. Five experiments, some with different collections of virus, showed that samples removed from this visible zone were 100–1000 times as infectious as samples removed 2 mm above this zone.

Extracts from infected plants heated at pH 7.0 or 8.0 for 1 hour at 40°C did not yield a visible zone, whereas solutions heated at pH 5.5 gave only a very faint zone. Heated extracts gave a visible zone only if they were made from the 2 or 3 youngest leaves of infected wheat. The normal constituents of old leaves seemed to be more resistant to heat denaturation. The term "denaturation" has been used here to describe the changes that occurred during heating at 40°; it is realized that some of these changes may have been due to enzyme action.

An extract heated 10 or 20 minutes at 40° at pH 6.0–6.5 did not give a visible zone, whereas one heated 30, 60, or 120 minutes did give a visible zone. Extracts heated 3 hours or more at 40° gave no visible zone.

In many experiments the crude extract was shaken with chloroform and/or *n*-butanol under a variety of experimental conditions (2, 13, 15). A few very clean zones were obtained after rate zonal centrifugation of treated extracts. However, these zones could not be consistently obtained. In general, the results obtained in this series of experiments were erratic, both as regards the survival of infectivity and the appearance of a visible zone after rate zonal centrifugation. Results were seldom as good as those obtained after heating for 1 hour at 40°C.

Those treatments that did not result in appearance of a visible zone were not investigated further: lack of a visible zone indicated that the treatment was not useful for purification. The ease with which a zone may be seen depends on the ratio of light scattered from the zone to that scattered from regions directly above and below, and is therefore a rough indication of the ratio of the concentration of particles in the zone to the concentration in the regions above and below.

Equilibrium zonal centrifugation.—When a sample of the visible zone obtained from rate zonal centrifugation was used for equilibrium zonal centrifugation, infectivity was associated with a visible zone that sedimented slightly faster than the green zone of chloroplast fragments, but was not completely separated from it. None of the normal plant constituents sedimented further than the virus, but some appeared to sediment as far. Additional centrifugation did not produce a complete separation. Many of the normal constituents did not sediment into the layer of sucrose solution containing 300 g/liter. These apparently had a low density. A complete separation of the virus from these components was obtained in this step. After equilibrium zonal centrifugation, the virus zone was usually 1.5 cm from the bottom of the ½ × 2-in. tubes, and 2.0 cm from the bottom of the 1 × 3-in. tubes.

Zone electrophoresis.—Zone electrophoresis at pH 7.8 of a sample from the visible virus zone obtained by rate zonal centrifugation resulted in 3 light-scattering zones. Infectivity was associated with the middle zone since samples removed from it were 10–100 times as infectious as samples removed to within 1–2 mm above or below this zone. The fastest zone was green, whereas the slowest zone was colorless and at least twice as wide as either of the other 2 zones. Neither the green zone nor the virus zone changed width appreciably as migration proceeded. The virus zone and green zone were not separated after electrophoresis at pH 6.0 or pH 7.0 with buffer containing 0.01M KCl and 0.005M potassium phosphate.

Purification of larger quantities.—The above experiments were all made with unconcentrated crude extracts. The most promising steps were selected and combined with 1 cycle of differential centrifugation to give a procedure for purification of larger amounts: 300–400 g of young leaves from diseased wheat were ground and the pulp mixed with an equal weight of 0.01M K_2HPO_4. After it was squeezed through cheesecloth, the extract was heated to 40°C over a burner and held 1 hour at 40°C in a water bath. The extract was then cooled and centrifuged 25 minutes at 20,000 rpm (mean R.C.F. 30,000 × G) in the No. 30 rotor. The pellet was discarded and the supernatant centrifuged 2½ hours at 30,000 rpm (mean R.C.F. 78,000 × G) in the No. 30 rotor. This pellet was suspended in 15 ml of 0.1M pH-7 phosphate buffer. Five ml was floated on each of 3 "rate" gradient columns and centrifuged 2 hours at 24,000 rpm in the SW-25.1 rotor to give a virus zone 2.5–3.5 cm deep. Five ml was removed from each tube at a depth of 3.5 cm and floated directly on an "equilibrium" gradient column in a 1 × 3-in. tube. After 7 ml of water had been floated on the virus solution, the tubes were centrifuged 7 hours at 24,000 rpm in the SW-25.1 rotor. About 1.7 ml was removed from the virus zone of each tube and 0.9 ml buffer added to the combined samples. The buffer and virus solution were thoroughly mixed and 1 ml floated in 1 leg of each of 6 U-tubes. Five hundred volts was ap-

TABLE 2.—*Stability of unpurified wheat streak mosaic virus at 40°C*

pH	Log_{10} concentration [a]		% surviving	
	Expt. 1	Expt. 2	4 hr.	1 hr. (calc.) [c]
5.5	1.20 ± 0.07	1.00 ± 0.07	0.7	28
6.0	1.19 ± 0.19 [b]	0.99 ± 0.06	0.7	28
7.0	1.71 ± 0.06	1.40 ± 0.06	1.9	37
8.0	0.72 ± 0.17 [b]	1.39 ± 0.06	0.6	27
Control at 0°C	3.05 ± 0.13 [b]	3.50 ± 0.06		

[a] Logarithm and standard deviation of the number of infectious particles per infection-initiating volume.
[b] Standard deviation corrected for a high value of χ^2.
[c] Calculated percentage that should have survived 1 hour if its destruction is a first-order reaction.

plied, to give 6–7 milliamperes per U-tube, and electrophoresis allowed to proceed for 8–12 hours. The virus zones were removed, diluted with an equal volume or more of buffer and centrifuged 2 hours at 36,000 rpm in the SW-39 rotor. The supernatant was discarded and the pellet taken up in an appropriate buffer. This final pellet was often slightly yellowish-green, even though the green zone had appeared to be completely separated from the virus zone during electrophoresis. All gradient columns were made from sucrose dissolved in the electrophoresis buffer, which was 0.01M KCl, 0.005M K_2HPO_4, and 0.0005M KH_2PO_4.

The over-all yields in this procedure, judged by infectivity assays, varied from 1 to 10 per cent. The purified virus was not weighed, but it is doubtful if more than 1 mg was obtained at a time. The final pellets obtained in ½ × 2-in. tubes were no more than 2–3 mm in diameter.

Stability of unpurified virus.—Four samples of crude extract from infected plants were adjusted to pH 5.5, 6.0, 7.0, and 8.0 and heated 4 hours at 40°C. Preliminary experiments had indicated that these conditions should give about 1 per cent survival. The infectivities of the samples were compared with that of a fifth sample, at pH 7.0, kept at 0°C. Results from 2 such experiments are shown in Table 2. More virus was lost in the second experiment than in the first, except for the samples heated at pH 8.0, which may be the result of substances in wheat extracts affecting the stability of the virus (see below). These substances may vary in concentration from time to time.

The last 2 columns in Table 2 show the average percentage of infectivity that survived 4 hours in the 2 experiments, and the calculated percentage that should have survived 1 hour if its destruction is a first-order reaction.

Effect of wheat sap on assay of purified virus.—In a preliminary experiment on its stability, a sample of purified virus heated 2 hours at 40°C after addition of healthy-wheat sap gave a higher infectivity titer than a control sample, unheated and inoculated without addition of wheat sap. The most plausible explanation appeared to be that wheat sap affected efficiency of inoculation, increasing the apparent titer, and experiments were designed to test the hypothesis. Two experiments were performed with preparations purified by the procedure given above (heating, differential centrifugation, zonal centrifugation, and zone electrophoresis). The sucrose was not removed from the final preparation. Samples of this preparation, which had a dilution of 30:1 (1 ml from 30 g leaves), were diluted 10-fold with 0.01M neutral potassium phosphate buffer or healthy-wheat extract. The latter was prepared by macerating wheat leaves in a mortar with twice their weight of 0.01M K_2HPO_4. The extract, of pH 6.2–6.5, was used without further treatment after being squeezed through cheesecloth. The virus samples were allowed to stand 1 hour at 2°C after the initial dilution, and then further diluted with 0.01M neutral potassium phosphate buffer or healthy-wheat extract, for inoculation into plants. In both experiments, the samples to which healthy-wheat sap was added before dilution with buffer had a titer 4 times as high as that of samples diluted with buffer only. Samples in which the entire dilution series was prepared with wheat sap gave an intermediate titer.

Stability of purified wheat streak mosaic virus.—Burger (7) reported that wheat streak mosaic virus purified by 1 cycle of differential centrifugation lost all infectivity after storage for 16 hours at 4°C. He further reported that this loss was prevented by adding 1 per cent glycerol, glucose, or sucrose, or 0.1 per cent gelatin, and suggested that the virus may be stabilized by similar materials in plant extracts. Loss of stabilizing factors might account for some of the low yields obtained in the present work; restoring stabilizers might increase yields. However, since none of the present purified preparations lost infectivity completely, an attempt was made to confirm the action of these stabilizing factors.

Six ml of a virus preparation (made by heating, differential centrifugation, zonal centrifugation, and zone electrophoresis) was diluted with 8 ml of 0.01M neutral phosphate buffer and centrifuged 2 hours at 36,000 rpm in the SW-39 rotor. The pellets were resuspended in 14 ml of the same buffer and the virus again centrifuged down. The pellet was resuspended in 1 ml of buffer. This preparation gave a negative Seliwanoff resorcinol test for fructose (11). This test gave a positive result when 0.01 per cent sucrose was added. Aliquots of this preparation were diluted with 4 volumes of 0.01M neutral phosphate buffer, with buffer containing 1.25 per cent sucrose, or with healthy-wheat extract. The healthy-wheat extract was prepared by macerating wheat leaves with twice their weight of 0.01M K_2HPO_4, filtering through cheesecloth, and adjusting to pH 7.0. The 3 virus solutions were heated at 40°C and samples were removed at 2 hours and 6 hours and cooled. Missing components were added to give all samples the same composition, except for active virus, when inoculated. Healthy-wheat extract heated 6 hours was added to the unheated virus sample. The results of the infectivity

TABLE 3.—*Stability of wheat streak mosaic virus purified by zonal centrifugations and electrophoresis in presence of buffer, 1% sucrose, and healthy-wheat extract*

Virus heated with	Log concentration [a]	
	2 hr. at 40°C	6 hr. at 40°C
0.01M phosphate	0.94 ± 0.07	0.44 ± 0.14 [b]
1% sucrose	0.65 ± 0.07	0.10 ± 0.06
Wheat extract	0.03 ± 0.06	−0.74 ± 0.07
Unheated control	0.83 ± 0.06	

[a] Logarithm and standard deviation of the number of virus particles per infection-initiating volume.
[b] Standard deviation corrected for high value of chi square.

assays are given in Table 3. The data indicate that 1 per cent sucrose had no effect on the stability of the purified virus, and that healthy-wheat extract decreased the stability of the virus.

Since the results of these experiments support conclusions exactly the opposite of those reached by Burger (7), a series of experiments were performed using his purification technique. Young wheat leaves infected with wheat streak mosaic virus were ground in a meat grinder and the sap squeezed from the pulp. Acetic acid was added to bring the extract to pH 4.9. After it had stood 1 hour at 2°C, it was centrifuged 1 hour at 3,600 G in the Servall Model-SPX centrifuge. The supernatant was adjusted to pH 6.0 with N NaOH and left overnight at 2°C. An aliquot was saved for infectivity assay and the rest was centrifuged 1 hour at 30,000 rpm in the No. 30 rotor. The pellet was resuspended in water (1 ml/100 g leaves), and 2 hours later this suspension was clarified by centrifuging 1 hour at 3,600 G in the Servall Model-SPX centrifuge. The supernatant was left overnight at 2°C, and 2 series of dilutions prepared in 10-fold steps. Distilled water was the diluent in 1 series. In the second series, healthy-wheat extract was used for the first dilution, and 0.01M neutral phosphate buffer for further dilutions. The unpurified crude extract and the clarified extract at pH 6.0, both of which had been stored at 2°C, were assayed at the same time as the concentrated, partially purified virus. The results are shown in Table 4.

According to these data the purified virus preparation was about 1/10 as infectious as the unconcentrated extract after clarification at pH 4.9 and readjustment to pH 6.0. The loss of infectiousness could be due to instability, losses in the purification procedure, or differences in efficiency of inoculation. Only half of the virus should have been sedimented during the hour's centrifugation at 30,000 rpm. The 10-fold loss reported here may be sufficient to explain Burger's results since he apparently inoculated dilutions covering only a 10-fold range and, furthermore, compared results of assays from inoculations made on different days. A 10-fold decrease under those conditions may have resulted in no infected assay plants.

DISCUSSION.—The wheat streak mosaic virus is not easy to purify, because of its low concentration and moderate instability. Some preparations obtained by a combination of zonal centrifugation and zone electrophoresis were quite pure as judged by the absence of other light-scattering zones in the electrophoresis. The yields were small and variable. Percentage yield as judged by infectivity assays without added wheat sap varied from 0.1 to 5 per cent. These measurements were probably low, since it was later shown that added wheat sap increased the apparent infectivity titer of purified virus. The apparent yield varied from 1 to 10 per cent in experiments where the purified virus was assayed after addition of healthy-wheat sap. There is no assurance that the assays of purified virus, even after addition of wheat sap, are completely comparable with those of unpurified virus. The results of the infectivity assays indicate that two thirds of the virus was lost during the initial heating for 1 hour at 40°C. In several experiments the pellet of the rate zonal centrifugation tube contained as much virus as the zone itself. A variable amount of virus was lost below the zone and in the pellet of the equilibrium zonal centrifugation tubes. Up to 70 per cent of the virus put on the equilibrium column was found below the zone; at other times this fraction had almost no virus. Incidental observations have indicated that this virus has a tendency to aggregate when a certain degree of purity is obtained. This may account for the variable losses in the equilibrium zonal centrifugation. Taking account of these losses, the expected yield would be 5–15 per cent. Further, smaller losses occur at each step because of inability to remove zones completely.

Gold, Houston, and Oswald (9) estimated, from particle-counting experiments, that infected wheat leaves contain 10–50 μg of wheat streak mosaic virus per g. Assuming an over-all yield of 10 per cent, 0.3–1.5 mg of purified virus should be obtained from 300 g of leaves. The final pellets obtained in the ½ × 2-in. tubes were no more than 2–3 mm in diameter, a size consistent with the expected yield of 1.5 mg or less.

Contrary to earlier reports, extracts from healthy wheat made the purified virus more unstable. On the other hand, healthy-wheat extracts increased the apparent titer of purified virus if added shortly before inoculations were made for infectivity assays. Thus the wheat extract has at least 2 effects, which may, at times, counteract each other.

TABLE 4.—*Infectivity assay after storage for 16–20 hours at 2°C of wheat streak mosaic virus purified by 1-cycle differential centrifugation*

Virus preparation	Log_{10} concentration [a]		
	Expt. 1	Expt. 2	Expt. 3
Crude extract	1.80 ± 0.09	1.47 ± 0.22 [d]	1.28 ± 0.29 [d]
Clarified extract at pH 6.0	1.43 ± 0.07	0.93 ± 0.08	1.34 ± 0.08
Purified virus [b]	0.04 ± 0.08	0.07 ± 0.07	0.19 ± 0.08
Purified virus [c]	0.36 ± 0.11	−0.10 ± 0.28	−0.43 ± 0.15 [d]

[a] Logarithm and standard deviation of the number of infectious particles per infection-initiating volume.
[b] Diluted with distilled water.
[c] Diluted with 0.01M potassium phosphate buffer after addition of healthy-wheat extract.
[d] Variance corrected for a high value of chi square.

In the various stability experiments it was assumed that the loss of virus and normal proteins was the result of heat denaturation. It is realized that this may not be the whole story, since enzymatic action may also have played a part. If the virus was destroyed in part by enzymatic action, the calculated percentage losses in Table 2 may not be valid. The present assay method is not adequate for determination of the kinetics of the heat destruction of this virus.

The data in Table 2 indicate that there was little difference in stability of the virus between pH 5.5 and 6.0. On the other hand, extracts heated at pH 5.5 gave no visible zone after rate zonal centrifugation, whereas those heated at pH 6.0 did. Two explanations may be advanced for this: the virus may be more highly aggregated at pH 5.5 than at pH 6.0; or more morphologically intact but noninfectious particles may be present after heating at pH 6.0 than after heating at pH 5.5.

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REACTION OF SWEET POTATO VARIETIES AND SEEDLINGS TO SOIL ROT [1]

W. J. Martin [2]

SUMMARY

Studies on varietal reaction to soil rot of sweet potato incited by *Streptomyces ipomoea* (Person and W. J. Martin) Waksm. & Henrici have shown rather clear-cut differences among varieties, selections, and breeding lines, ranging from very striking susceptibility (in Unit 1 Porto Rico, the principal variety planted in Louisiana) through some degree of tolerance (in the Heartogold and Acadian varieties) to considerable resistance (in certain other selections and breeding lines).

Soil rot, incited by *Streptomyces ipomoea* (Person and W. J. Martin) Waksm. & Henrici,[3] annually causes heavy losses to the sweet potato industry in Louisiana. During the last decade it has been by far the most important field disease of sweet potato in the state. A practical field control developed in 1937–45 [4] became less attractive as sulfur cost increased and the disease spread to more diverse soil types, where lowering the pH was less desirable from the standpoint of succeeding crops.

Since 1948, observations and experiments have been made on the relative reaction to soil rot of sweet potato varieties, breeding lines, and seedlings [5] to determine

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[3] Person, L. H., and W. J. Martin. 1940. Soil rot of sweet potatoes in Louisiana. Phytopathology 30: 913-926.

[4] Person, L. H. 1946. The soil rot of sweet potatoes and its control with sulfur. Phytopathology 36: 869-875.

[5] Appreciation is hereby expressed to members of the Horticulture Department, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, who furnished many of the sweet potato selections used in these studies, and to C. E. Steinbauer, Plant Industry Station, Beltsville, Maryland, who furnished the Plant Introduction selections.

TABLE 1.—*Mean yield, in bushels per acre, and mean rating of vine growth of 6 sweet potato varieties in plots heavily infested with Streptomyces ipomoea at Sunset, Louisiana, 1955-56*

Variety	1955 Experiment			1956 Experiment			Combined 1955 & 1956		
	Bushels/acre [a]		Vine growth rating [b]	Bushels/acre [a]		Vine growth rating [b]	Bushels/acre [a]		Vine growth rating [b]
	U. S. No. 1 & 2	Total		U. S. No. 1 & 2	Total		U. S. No. 1 & 2	Total	
Unit 1 Porto Rico	39.0	74.8	1.3	0.0	4.8	1.0	19.8	39.8	1.13
Darkskin Goldrush	83.8	169.3	2.0	37.5	153.3	2.3	60.8	161.5	2.13
Earlyport	53.3	92.8	1.3	0.0	12.3	1.0	26.8	52.8	1.13
Allgold	66.0	161.0	3.0	5.5	63.0	2.0	36.0	112.3	2.50
Heartogold	162.3	235.8	3.0	125.5	323.3	3.0	144.0	279.8	3.00
Acadian [c]	128.0	195.3	3.0	81.5	244.3	3.0	105.0	220.0	3.00
LSD 5%	35.5	52.9	0.38	50.0	84.4	1.30	29.8	40.5	0.58
1%	48.2	71.9	0.51	67.9	114.6	1.77	40.5	55.0	0.68

[a] Mean of 4 replicates with 1-row plots 40 ft. long.
[b] Vine growth in each plot about 7 weeks after planting was rated as follows: 1, poor; 2, fair; 3, good.
[c] Formerly called La. 1-13.

the practicability of breeding for resistance to the disease. This paper records the resulting information and data.

Early experiments revealed that the Heartogold variety had considerable tolerance to soil rot, producing a good root system and good yields even in heavily infested soils. Enlarged roots of Heartogold, however, developed severe lesions (Fig. 1B), and the resistance in this variety appeared to be due to its characteristic of producing an abundant root system rapidly, thereby escaping the severe early effects of rootlet rot so characteristic in the very susceptible Unit 1 Porto Rico variety.

Attempts to develop a fairly reliable greenhouse method of evaluating the reaction of sweet potato selections to soil rot have not been successful so far. Even when using the susceptible Unit 1 Porto Rico, conditions of moisture and temperature must be carefully controlled to obtain severe rootlet rot in sterilized soil heavily infested with *S. ipomoea*.[3] Therefore, it has been necessary to rely largely on field experiments in infested soil for information on varietal reaction.

METHODS.—Data were obtained from replicated, randomized field plots planted during May to ensure maximum disease development. Rating of vine growth was made about 7 and, in some cases, 11 weeks after planting. Plots were kept free of weeds up to the time of rating, then left undisturbed until harvested. At harvest time all roots in each plot were sorted into the different grades and weighed. Yield data were converted to bushels per acre.

RESULTS.—Highly significant differences in vine growth were found among varieties and selections in most of the experiments made in heavily infested soil (Tables 1 and 3). Outstanding in this respect were the Heartogold and Acadian varieties and Louisiana seedlings 2-61 and 3-7 (Tables 2 and 3). These varieties and seedlings were outstanding also in yield of marketable roots (U. S. grades No. 1 and 2). Among

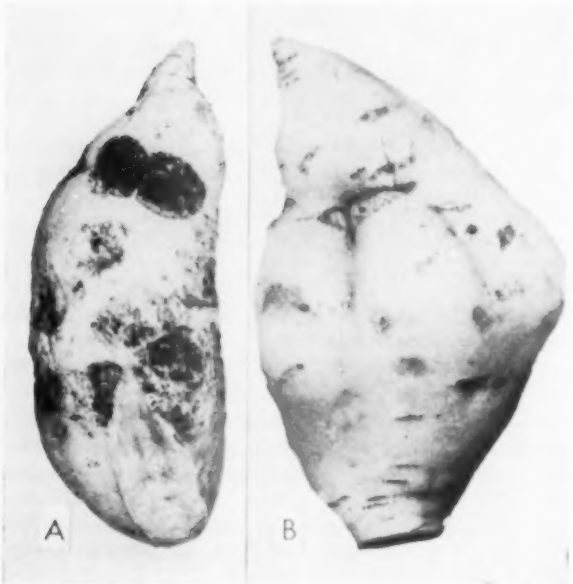

FIGURE 1. Sweet potato roots affected with soil rot. A) Acadian variety. B) Heartogold variety.

TABLE 2.—*Mean yield in bushels per acre and mean rating of vine growth of sweet potato varieties and seedlings in plots lightly infested with Streptomyces ipomoea at Arnaudville, Louisiana, 1956*

Selection	Bushels/acre [a]			Vine growth [b]
	U. S. No. 1	U. S. No. 2	Total	
Unit 1 Porto Rico	140.8	62.8	342.0	3.0
Darkskin Goldrush	157.8	52.5	316.0	2.8
Earlyport	126.3	42.0	254.0	2.5
Heartogold	171.5	54.0	350.8	3.0
Acadian	209.5	41.3	338.0	2.8
La. 2-61 seedling	235.5	55.5	369.8	3.0
La. 3-7 seedling	92.3	58.5	282.3	3.0
La. 3-47B seedling	87.5	30.8	258.0	2.8
LSD 5%	95.5	n.s.[c]	n.s.[c]	n.s.[c]
1%	n.s.[c]	n.s.[c]	n.s.[c]	n.s.[c]

[a] Mean of 4 replicates with 1-row plots 40 ft. long.
[b] Vine growth was rated as follows: 1, poor; 2, fair; 3, good.
[c] Differences not significant.

TABLE 3.—*Mean yield in bushels per acre, mean rating of vine growth, and root lesions of sweet potato varieties and seedlings in plots heavily infested with Streptomyces ipomoea at Sunset, Louisiana, 1957*

Variety and seedling	Bushels/acre [a] U. S. No. 1 & 2	Bushels/acre [a] Total	Vine growth [b] At 7 weeks	Vine growth [b] At 11 weeks	Root lesions at harvest [c]
Experiment 1					
Unit 1 Porto Rico	35.0	98.8	1.8	2.0	2.8
Acadian	152.0	318.3	3.0	2.8	1.8
La. 1-80 seedling	67.8	161.3	2.0	2.0	2.3
La. 1-183 seedling	5.0	38.3	1.0	1.5	3.0
La. 2-61 seedling	88.0	186.5	3.0	3.0	1.0
La. 3-80 seedling	0	7.0	1.0	1.8	3.0
La. 3-151 seedling	94.0	215.0	2.3	2.5	2.0
LSD 5%	51.8	78.9	0.87	n.s.[d]	0.44
1%	69.8	106.2	1.17	n.s.[d]	0.59
Experiment 2					
Acadian	134.3	298.8	2.3	3.0	1.8
La. 3-7 seedling	129.5	228.0	2.5	3.0	1.0
La. 4-12 seedling	0	14.5	1.3	1.3	3.0
La. 4-142 seedling	30.0	97.0	1.5	2.0	2.0
La. 4-148 seedling	21.8	72.3	1.8	1.0	2.3
La. 5-93 seedling	6.0	41.5	1.0	1.5	2.8
La. 5-94 seedling	40.0	134.5	2.8	2.8	2.5
LSD 5%	49.6	91.7	0.64	0.71	0.71
1%	66.8	123.4	0.87	0.96	0.95

[a] Mean of 4 replicates in each experiment, with 1-row plots 40 ft. long in experiment 1 and 20 ft. long in experiment 2.
[b] Vine growth was rated as follows: 1, poor; 2, fair; 3, good.
[c] Roots were rated for lesions as follows: 1, slight, 2, moderate; 3, severe.
[d] Differences not significant.

the other varieties both Darkskin Goldrush and Allgold were significantly higher in total yield in some tests than Unit 1 Porto Rico and Earlyport, both of which yielded very poorly in the heavily infested soils.

Great variations in yield were evident from year to year in the same infested soil (Tables 1 and 3). Yields of most varieties were somewhat higher in the wet growing seasons of 1955 and 1957 than in the relatively dry season of 1956. An exception was the resistant seedling La. 2-61, which yielded poorly in 1957 for reasons other than apparent disease. Soil rot is most severe during dry seasons.

Reaction of the Acadian variety to soil rot is considered similar to that of Heartogold, with somewhat less tolerance to the disease but with the same characteristic of producing abundant roots rapidly after setting of the plants. Lesions developed abundantly on roots of the Acadian variety (Fig. 1A), with slightly less tendency toward the early development of lesions that results in deep constrictions of the root in other varieties. The loss percentage from severe infection on enlarged roots of the Acadian variety is indicated in Table 4 under the "Acadian-untreated" column. Yield of U. S. grades No. 1 and 2 roots (relatively free of lesions) was 96.5 bushels per acre, whereas yield of roots culled (because of lesions) from U. S. grades No. 1 and 2 was 64.7 bushels per acre. Thus a loss of about 40 per cent of marketable yield from soil rot lesions is indicated in the Acadian variety grown in heavily infested soils.

The seedlings La. 2-61 and La. 3-7 are considered more resistant than Heartogold and Acadian to the development of soil rot lesions on roots.

Seven U. S. Department of Agriculture Plant Introduction selections planted in 1957 in 4-replicated plots in heavily infested soil failed to show any striking tolerance to soil rot. These were P. I. 207705, 208645, 208804, 208885, 208887, 208890, and 208891. However, indications of a high type of resistance were found in an observational plot in 1957 planted with single-row plots of 18 P. I. selections and 15 Louisiana seedlings. P. I. 208883 produced a high yield of lesion-

TABLE 4.—*Mean yield of 2 sweet potato varieties, in bushels per acre, of U. S. grades No. 1 and 2 roots, of roots in size range of U. S. grades No. 1 and 2 but culled because of soil rot lesions, total yield (including all culls), and mean pH values in plots heavily infested with Streptomyces ipomoea at Arnaudville, Louisiana, 1957*

Varieties and treatment [a]	Bushels/acre [b] U. S. No. 1 & 2	Bushels/acre [b] Cull No. 1 & 2	Bushels/acre [b] Total	pH values
Unit 1 Porto Rico, untreated	0.9	8.2	15.0	6.8
Unit 1 Porto Rico, treated	17.7	15.7	48.8	5.5
Acadian, untreated	96.5	64.7	201.3	6.9
Acadian, treated	125.2	29.3	192.5	5.5
LSD 5%	22.2	27.3	30.2	
1%	30.2	37.2	41.1	

[a] Treated plots received sulfur at the rate of 600 lb. per acre in the fall of 1956. Sulfur applied was insufficient to reduce pH to the desired 5.0 level for control of soil rot.
[b] Mean of 6 replicates, with 3-row plots 50 ft. long.

free roots, and P. I. 219660 produced a high yield with relatively few lesions. In the same test, La. 3-131 and La. 4-136 also had good yields with low incidence of disease.

The data given clearly demonstrate that there is a commercially valuable degree of tolerance to soil rot among sweet potato varieties. It is also suggested that, since there is a fairly high degree of resistance in seedlings and, possibly, some P. I. selections, a thorough search should be made among the numerous available sweet potato selections for breeding lines with higher resistance to the disease.

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FERTILIZERS INFLUENCE THE INCIDENCE OF TOMATO INTERNAL BROWNING IN THE FIELD[1]

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SUMMARY

Late inoculation with Boyle and Wharton's C-IB-Tob strain of tobacco mosaic virus alone did not induce internal browning (IB) of tomatoes in the field. When nutritional levels of potassium and phosphorous were varied, IB was significantly worse in virus-inoculated plots. High potassium significantly reduced IB in virus-inoculated plots; high phosphorous had little effect. IB showed in more than 1 picking from the same plant. Some plants showed IB, then were free of IB for 1 or more pickings, then showed IB again. These skips are an apparent contradiction of the shock reaction theory of Boyle and Wharton. The contradiction is best resolved by Taylor's hypothesis, that IB may result from any cause that restricts the movement of sugars into the developing fruit.

Tomato internal browning (IB), a chlorotic and necrotic ripening disorder of tomato fruits, has recently been described by Boyle and Wharton (3) as a shock reaction to a virus infection of a previously virus-free plant. These workers could find no evidence that fertilizers influence the incidence of internal browning in the field. Taylor (7), working with carefully controlled greenhouse experiments, found the incidence of the disease in virus-infected plants to be greatly influenced by nitrogen and boron nutrition. From his leaf and fruit analyses, Taylor believes that IB is the result of interference with the movement of reducing sugars to the developing fruits. He postulates that any conditions that restrict the movement of reducing sugars may bring about IB. Kidson and Stanton (5), in New Zealand, and Bewley and White (2), in England, working with what they call "blotchy-ripening" of tomatoes, reported that high potassium fertilizing reduces the incidence of the disease. Kidson and Stanton concluded that the disease in New Zealand is the result of an abnormally low organic matter content in the fruit. Neither Kidson and Stanton nor Bewley and White investigated the relation of virus infection to their tomato fruit disorders. Whether "blotchy-ripening" is identical with the American IB is not known. Stoner and Hogan (6) could find no virus involved in the production of "graywall" in Florida. The symptoms of graywall appear to be identical with those of internal browning.

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The author is deeply indebted to A. D. McDonnell and his assistants, M. R. Siegel and W. J. Birmingham, for their excellent and conscientious efforts during the course of these experiments.

The experiments described here were done in 2 years, 1956 and 1957. The 1956 experiment was designed to test the importance of virus alone as the cause of IB in Connecticut. The 1957 experiment attempted to relate potassium and phosphorous soil applications and virus inoculation to the incidence of IB.

1956 EXPERIMENT.—The 1956 experiment was carried out on adequately and uniformly fertilized land. Sioux variety seedlings were set out, 4 plants per plot and 16 plots per block, in 4 randomized blocks. The virus inoculum, kindly furnished by Dr. John S. Boyle, was the strain designated by him as C-IB-Tob (3). Four of the treatments received virus. The plants in 3 of the virus treatments were inoculated by the method of Boyle and Wharton, by injection with a hypodermic syringe into the peduncle of the first cluster when the largest fruits on the cluster were ½–¾ grown. The plants in the fourth virus treatment were inoculated at the same time, by injection into their stems. There were no visible foliage symptoms of virus in the field at the time of inoculation. Within 10–14 days, typical chlorotic foliage symptoms appeared on the inoculated plants. The virus spread through the uninoculated plots during the rest of the season.

When the fruits were fully ripe on the first 2 clusters, they were examined externally and internally for IB. Of the 3177 fruits examined, only 35 showed IB, only 2 of which were from the inoculated plots.

1957 EXPERIMENT.—To impose appreciable differences in nutrient levels, the 1957 experiment was carried out on a piece of land that, at the start, had potassium and phosphorous in amounts just sufficient to support plant growth. The entire area to be used re-

TABLE 1.—*Percentage of total fruits that showed internal browning (1957)*

Treatment	Replicate						Average
	1	2	3	4	5	6	
VK	6.7	14.4	9.3	8.8	9.3	6.4	9.2
VP	16.7	23.2	32.1	11.4	13.6	10.1	18.3
VPK	9.0	14.1	10.5	17.2	11.0	13.3	12.5
V	12.4	26.0	18.2	14.9	15.2	7.5	15.9
K	4.6	4.4	5.6	0.0	9.3	2.7	4.5
P	1.0	9.6	7.2	3.3	1.7	11.4	5.4
Check	1.3	4.3	2.8	1.2	6.1	0.0	2.6

ceived ammonium nitrate at the rate of 85 lb. of nitrogen per acre. The individual plots, each 7 × 10 ft., were arranged in 6 randomized blocks, 7 treatments per block. Plots for low potassium and low phosphorous received no further fertilizing. Plots for high potassium received KCl at a rate equivalent to 170 lb. of K_2O per acre. Plots for high phosphorous received superphos at a rate equivalent to 170 lb. of P_2O_5 per acre. The virus inoculum, taken from the 1956 experiment, was carried over on tomatoes in the greenhouse. Sioux variety tomato seedlings, 6 plants per plot, were set out within a few days after the fertilizers were applied. The plants in the virus treatments were inoculated with a hypodermic syringe when the largest fruits on the first clusters were ½–¾ grown. The inoculum was injected into the peduncles of the first clusters on each plant in the virus treatments. There were no visible foliage symptoms of virus in the field at the time of inoculation. The foliage symptoms appeared in the inoculated plants 10–14 days after they were inoculated.

The 7 treatments in the 1957 experiment were VK (virus—high-K—low-P), VP (virus—low-K—high-P), VPK (virus—high-K—high-P), V (virus—low-K—low-P), K (no-virus—high-K—low-P), P (no-virus—low-K—high-P), check (no-virus—low-K—low-P). No attempt was made to prevent the natural spread of the virus. The fruits were harvested by entire clusters as they ripened, and carefully examined, both externally and internally, for IB. The first clusters, which included those directly inoculated with IB, were harvested on September 3; the second clusters on September 6; the third clusters on September 16; and the fourth clusters on September 19. A total of 4569 fruits were examined from the 252 plants.

IB symptoms were severe at no time, never exceeding the 2 rating on the scale used by Boyle and Wharton (3). The percentages of IB in the total fruits picked in all 4 pickings from each treatment are given in Table 1.

These data were analyzed using Wilcoxon's ranking methods (9). For the entire experiment the treatment differences were significant at the 0.003 level. Individual treatment differences were compared at the 0.05 level. The analysis showed that all virus-inoculated plots had significantly more IB than the comparable non-inoculated plots, regardless of fertilizer treatment. VK had significantly less IB than all other virus-inoculated plots. There was no significant difference between the amounts of IB in VPK, VP, and V. There was also no significant difference between the amounts of IB in P, K, and the check.

The incidence of IB through the season in each treatment is shown in Table 2. In every treatment the disease generally became progressively more prevalent with each successive picking. In almost all cases there was less IB in the first picking than in any of the succeeding pickings. The non-inoculated check had as much IB in the first picking as did the comparable V treatment, in which the first clusters had been inoculated.

The distribution of IB throughout the season by individual plants and by pickings is a characteristic of the disease. In 1957, separate records for each picking of the total number of fruits picked and the total number of fruits with IB were kept for each of the 252 plants in the experiment. In totals for the season, 68 per cent of the plants showed IB at least once, 30 per cent showed IB 2 or more times, 6 per cent showed IB 3 times, and 1 plant showed IB in all 4 pickings. The virus-inoculated plots generally had a higher percentage of plants showing IB at least once, and also a larger percentage that showed IB more than once; 4.6 per cent of the plants showed IB, then were clean for 1 or more pickings before showing IB again.

DISCUSSION.—These experiments demonstrate that the incidence of IB in the field can, indeed, be influenced by fertilizing. The 1957 experiment shows high potassium can alleviate IB in virus-inoculated plots. That high potassium would also reduce the incidence of blotchy ripening in greenhouses was also found by Bewley and White (2) and by Kidson and Stanton (5). Although Taylor (7) compared the influence of high potassium with that of medium potassium on the incidence of IB, he had no IB develop at either level of potassium in his comparable treatments. His results regarding potassium, therefore, were inconclusive. The role of phosphorous in connection with IB is not clear. The 1957 experiment indicates that high phosphorous tends to obscure the beneficial effect of high potassium.

Two conditions made it possible to show the effect of nutrients on IB in the field. One was the choice of soil low in potassium and phosphorous, so that barely adequate levels could be compared with more than adequate levels. The second condition was the very dry growing season, which made for very much less than usual leaching of potassium from the soil. Thus, the nutritional differentials remained fairly constant

TABLE 2.—*Percentage of internal browning in each treatment at each picking (1957)*

Treatment	Date of picking			
	9/3	9/6	9/16	9/19
VK	5.4	7.4	12.7	14.5
VP	3.9	12.9	28.2	22.0
VPK	6.9	8.0	11.5	15.0
V	2.5	13.9	22.9	21.1
K	1.6	1.5	3.3	10.1
P	0.8	2.0	9.6	9.3
Check	2.4	1.6	3.4	5.4

throughout the growing season, particularly at the critical periods of fruit development.

These experiments also indicate certain things about the role of virus in the incidence of IB. The 1956 experiment shows that virus infection alone will not result in severe IB. The 1957 experiment demonstrates that, when IB does occur, it is definitely worse where virus is inoculated. At least 2 portions of the 1957 data are in apparent contradiction to the shock-reaction theory of Boyle and Wharton (3). The last set of fruit had the most IB, yet these clusters were at early flower or younger when the plants were inoculated. Boyle and Wharton indicated that, in order for the virus to produce IB, the cluster to be affected must be older than early flower when the plant is inoculated. The second apparent contradiction to their shock-reaction theory is suggested by the "skips," i.e. plants that had IB in a cluster, followed by pickings with no IB, then showed IB again in a later cluster. If the shock reaction is dependent on virus invasion of a previously virus-free plant at a critical period of fruit development, then there should be no skips.

These data confirm that virus infection may have an important role in producing IB. The 1956 data, however, show that virus infection by itself is not the only factor responsible for IB in the field, even when virus invades the developing fruit at the critical period proposed by Boyle and Wharton. This observation confirms Taylor's results in the greenhouse. Whether IB may be present without virus, as it appears to be in Florida, cannot be determined from these experiments.

The most reasonable hypothesis for the appearance of IB is Taylor's (7) suggestion that it results from a sugar deficiency of the developing fruit. Using as his basis not only his own work but also that of Kidson and Stanton (5), Taylor suggested that "IB can result from a sugar deficiency of the developing fruit arising from separate and distinct causes acting independently or in conjunction with one another." If this assumption is accepted, then potassium influences IB by influencing the production or translocation of carbohydrates. Potassium may possibly influence carbohydrates either directly, as a regulator in the plant, or indirectly, by possibly influencing the multiplication of tobacco mosaic virus, which in turn has been shown to reduce carbohydrates (4). Two items indicate that the effect is direct rather than indirect. First, potassium nutrition appears to have little effect on the multiplication of tobacco mosaic virus (1, 8). Second, the potassium effect, both on blotchy-ripening and on the correlated carbohydrate content of the fruit, was also noted by Bewley and White and by Kidson and Stanton, who made no mention of virus. The level of available potassium, then, may influence the incidence of IB by being one of the factors controlling the amount of sugar or carbohydrates entering the developing fruit.

At present, it appears likely that the incidence and severity of IB may be reduced by taking measures to prevent tobacco mosaic virus from invading healthy tomato fields during fruit development, and by sidedressing with potassium at the time of first fruit set.

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THE INHERITANCE OF RESISTANCE TO BEAN VIRUS 2 IN PISUM SATIVUM [1]

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SUMMARY

The inheritance of resistance to bean virus 2 in *Pisum sativum* L. was studied by crossing 2 susceptible varieties, Perfected Wales and Thomas Laxton, to each of 4 resistant varieties, New Era, Pride, Wisconsin Early Sweet, and Wisconsin Perfection. Reciprocal F_1 hybrids, F_2 bulk, and F_3 and F_4 progenies from, respectively, single F_2 and F_3 plants of these 8 crosses were tested for reaction to bean virus 2 in the greenhouse. Results indicated that resistance was controlled by a single recessive gene. Observed deviations from the predicted 3:1 ratio in some of the F_2 bulk and some of the segregating (heterozygous) F_3 and F_4 progenies were attributed to a probable delayed symptom expression in the susceptible heterozygotes.

Pea mosaic, incited by bean virus 2, is recognized as a widespread virus disease of canning peas (*Pisum sativum* L.) in Wisconsin (2). In 1934, Pierce (5) reported a Perfection-type pea variety to be resistant to bean virus 2. Because of the large acreage of this type of canning pea in Wisconsin, Hagedorn (1) studied the reaction of many commercial varieties to determine if they were resistant or susceptible to bean virus 2. The resistance of Perfection-type peas as a group was confirmed, but a number of the newer strains in which earliness had been incorporated were susceptible, which was attributed to the use of a susceptible parent in the crosses made to incorporate earliness. Because of the importance of the disease and the evident inattention to resistance in the development of new varieties, the following investigation was undertaken to determine the mode of inheritance of resistance to bean virus 2 in *P. sativum*.

MATERIALS AND METHODS.—Pea isolate 1 of bean virus 2, described by Hagedorn and Walker (2), was used in all of the experiments. It had been maintained on sweet clover (*Melilotus* spp.) culture plants in the greenhouse. The virus was transferred to Perfected Wales, a susceptible variety, and was cultured in this variety and sweet clover throughout the experiments.

The symptoms on Perfected Wales in the greenhouse were typified by a distinct vein-clearing, about 10–14 days after inoculation, and chlorosis of tissue between the veins and patches of normal green tissue scattered irregularly over the leaf and stipule surface, about 14–16 days after inoculation. No stunting, necrosis, or pod and stem symptoms were observed.

Four resistant varieties (Wisconsin Perfection, Wisconsin Early Sweet, Pride, and New Era) were crossed reciprocally to 2 susceptible varieties (Perfected Wales and Thomas Laxton). Reciprocal F_1 populations, bulked F_2 populations, and F_3 and F_4 progenies of, respectively, single F_2 and F_3 plants were tested in the greenhouse for reaction to bean virus 2. Each filial generation tested was derived from uninfected parental plants. Check plants of both resistant and susceptible varieties were included in all tests.

The peas were grown in 6-in. pots containing sterilized compost soil. All experiments were conducted in the greenhouse at 15–20°C. Inoculations were made about 12–15 days after planting. Plants uninfected after the first inoculation were reinoculated 2–3 weeks after the first inoculation.

Diseased culture plants were ground through a fruit juicer to extract the sap, which was used in original concentration or diluted about 1:1 with distilled water. To facilitate infection, carborundum powder was dusted on the leaflets of healthy plants to be inoculated. The first inoculation consisted of rubbing infective juice on all of the fully expanded leaflets (usually 4) with a glass spatula or porcelain pestle. In the second inoculation only the apical or youngest 2–3 fully expanded leaves (about 4–6 leaflets) were rubbed.

EXPERIMENTAL RESULTS.—All 8 F_1 hybrids and their reciprocals were completely susceptible to bean virus 2. A total of 200 plants were tested, with an average of 12.5 plants per F_1 family. Some families had as many as 37–39 plants.

Table 1 gives the reactions of the bulk F_2 populations to bean virus 2. Since the F_1 crosses indicated no cytoplasmic effect, the reciprocal crosses were pooled. Chi-square values (based on the hypothesis of 3 susceptible to 1 resistant) were greater than the 1 per cent level in the Perfected Wales × Wisconsin Perfection and Thomas Laxton × Wisconsin Perfection crosses. The cross Perfected Wales × New Era approached the 1 per cent probability level. The remaining 4 crosses satisfactorily fit the ratio of 3 susceptible to 1 resistant.

The reaction to bean virus 2 of the F_3 and F_4 progenies, from single F_2 and F_3 plants, respectively, is given in Table 1. Because of adverse growing conditions, less than 10 seeds were obtained from a number of plants. With a monogenic character, a minimum of 10 plants per progeny are needed in the F_3 and F_4 progeny tests for distinguishing (with odds of 19:1) between the homozygous dominant and the heterozygous progeny (4). A minimum of 3 plants per progeny are needed for identifying (with the same odds) the homozygous recessives.

On this basis, only progenies with 4 or more plants were classified, and the susceptible and segregating classes were pooled. The expected ratios for the F_3 progenies would then be 3 susceptible segregating to 1

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resistant progeny. For the F_4 progenies, the expected ratio would be 5 susceptible segregating to 3 resistant progenies.

All crosses fit the predicted ratios, except 2 of the F_4 progeny populations of the crosses Perfected Wales × Pride and Thomas Laxton × New Era. The number of progenies tested in these crosses was 38 and 40, respectively. Larger populations would have been preferred.

The reactions of the segregating (heterozygous) F_3 and F_4 progeny classes to bean virus 2 are shown in Table 1. Only 3 crosses segregated in the expected ratio of 3 susceptible to 1 resistant.

Discussion.—All deviations from the predicted 3:1 ratio were due to a deficiency in the virus-susceptible classes. Circumstances that might account for this deficiency are: 1) selection was against the susceptible allele because of linkage with either a lethal seedling or ovule factor or with the short internode allele (3, 6); 2) the inoculation technique resulted in too many escapes; and/or 3) there was a delayed symptom expression in the heterozygotes (7).

Considering the first factor, all the varieties used in the experiment were "well tested" and widely grown varieties, and were presumed genetically stable. The nature of these varieties limits any presumption that lethals were present. If lethals were not present, the natural mutation rate(s) of the lethal locus (loci) plus linkage of this mutated allele(s) with the virus allele would have to account for the deficiency. The probability that this occurred is so small that this factor may very likely be eliminated from consideration. Other data, not reported here, indicated that the short internode allele was not linked with the bean virus 2 gene.

The possibility of a poor inoculation technique was recognized early in the investigation. The varieties Perfected Wales and Thomas Laxton were used as susceptible check varieties throughout all experiments. Susceptible check plants were rubbed—about 15 at the beginning and another 15 at the end of each inoculation. Over a 2-year period the susceptible check plants, with 1 exception (Table 2), were always infected in 1 inoculation. This indicated that the virus was present throughout each inoculation period. Considering the succulent nature of the pea leaflet and the use of carborundum as an abrasive, it seems unlikely that the virus was not into the plant. Leaf-cell injury at time of inoculation might have limited the substrate, thereby preventing the virus from becoming established. However, no hypersensitive reaction was observed. It was concluded that the inoculation technique could not be considered as a major factor contributing to the deficiency of susceptible plants.

After completion of the above experiments, Yen and Fry (7) reported on the inheritance of immunity to pea mosaic virus in pea varieties grown in New Zealand. They concluded that immunity is controlled by a single recessive gene. However, the susceptible heterozygote was noted to be only partially tolerant to infection, as typified by a delay in symptom expression. The possibility that the heterozygote conditioned a delayed response was not considered during the course of our experiments. Although incubation periods were not recorded, observations made on the F_1 and F_2 bulk populations indicated that this same phenomenon was probably manifested in our experiments.

In some of the F_1 crosses, 2 inoculations were required to infect all plants. Since these plants were heterozygous with respect to the alleles in question, the possibility exists that the first reading was made before the susceptible reaction was expressed. In some of the F_2 bulk populations, data were recorded as to the number of days from inoculation until symptoms were read (Table 2). If the heterozygote did condition a delayed response, the first reading would be expected to show a predominant number of resistant plants, or even a "reversed" ratio, of 1 susceptible to 3 resistant

Table 1.—*Reaction of pea crosses to bean virus 2*

Cross [a]	Susceptible	Resistant	Chi Square [b]
Bulk F_2	Number of Plants		3:1
(PW × NE)	135	61	3.919*
(PW × P)	94	29	0.133
(PW × WES)	66	33	3.667
(PW × WP)	121	107	**
(TL × NE)	109	51	4.033*
(TL × P)	117	46	0.902
(TL × WES)	167	45	1.610
(TL × WP)	89	101	**
F_3 progeny from single F_2 plants	Number of Progeny		3:1
(PW × NE)	72 [c]	17	1.652
(PW × WES)	170	61	0.244
(PW × WP)	106	33	0.177
(TL × WP)	89	35	0.688
F_4 progeny from single F_3 plants	Number of Progeny		5:3
(PW × P)	16 [c]	22	6.744**
(PW × WES)	30	23	0.768
(PW × WP)	41	25	0.578
(TL × NE)	19	21	3.840*
(TL × P)	51	21	2.133
Segregating (heterozygous) F_3 and F_4 progeny	Number of Plants		3:1
(PW × NE) F_3	272	149	**
(PW × P) F_3	33	59	**
(PW × P) F_4	18	9	1.000
(PW × WES) F_3	541	253	**
(PW × WP) F_3	224	197	**
(PW × WP) F_4	43	160	**
(TL × NE) F_3	57	34	7.417**
(TL × NE) F_4	84	48	9.091**
(TL × P) F_3	70	29	0.973
(TL × P) F_4	151	75	8.077**
(TL × WES) F_4	61	24	0.435
(TL × WP) F_3	222	172	**

[a] PW, Perfected Wales; P, Pride; WES, Wisconsin Early Sweet; NE, New Era; WP, Wisconsin Perfection; TL, Thomas Laxton.

[b] * = Chi Square significant at the .05 level. ** = Chi Square significant at the .01 level.

[c] Susceptible class includes both completely susceptible progeny and segregating (heterozygous) progeny.

TABLE 2.—*Number of days between inoculation with bean virus 2 and symptom classification for F_2 bulk pea populations*

Cross [a]	Inoculation number [b]	Days [c]	Number of Plants	
			Susceptible	Resistant
(PW × NE)	1	26	68	128
	2	63	135	61
(PW × WES)	1	25	43	56
	2	67	66	33
(PW × P)	1	21	86	37
	2	49	94	29
(PW × WP)	1	21	62	166
	2	49	121	107
(TL × NE)	1	21	71	89
	2	47	109	51
(TL × WES)	1	14	139	73
	2	44	167	45
(TL × P)	1	15	66	97
	2	38	117	46
(TL × WP)	1	14	49	141
	2	38	89	101
Checks				
PW	1	25	15	0
PW	1	14	2	14
	2	28	16	0
NE	1	25	0	15
NE	1	14	0	14
TL	1	21	16	0

[a] See Table 1 for varietal abbreviations.
[b] Number of times inoculated.
[c] Number of days from first inoculation until symptoms read.

plants. In 3 of the crosses, Thomas Laxton × Wisconsin Perfection, Perfected Wales × Wisconsin Perfection, and Perfected Wales × New Era, this "reversed" ratio was observed. Most of the other crosses had a predominant number of resistant plants at the first reading.

Yen and Fry (7) also reported that the heterozygote could be distinguished by the type of symptom expression. In the present experiments, except the crosses involving Wisconsin Perfection as a parent, symptom expression in all crosses was the same as that described for Perfected Wales in the greenhouse. In the Wisconsin Perfection crosses, the early vein-clearing symptoms were similar to those of Perfected Wales. However, the mottle or mosaic symptom was generally less discernible than in the other crosses or checks. This could account for the deviation from the 3:1 ratio in the crosses involving Wisconsin Perfection. Since the scope of the experiment prohibited testing all crosses at one time, they were tested successively over a period of 3 greenhouse seasons. It is not known whether this difference in symptom expression in the Wisconsin Perfection crosses was due to plant age and environment (8) or to modifying genes affecting symptom type.

In conclusion, it was felt that the results of the tests from the F_3 and F_4 progenies, respectively, of single F_2 and F_3 plants were conclusive enough to substantiate the hypothesis that resistance is monogenic and recessive in all the resistant varieties used in this experiment. The discrepant results in the segregating populations were probably due to the phenomenon of delayed symptom expression in the heterozygote.

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RESPONSES OF LIME AND SOUR ORANGE SEEDLINGS AND FOUR SCION-ROOTSTOCK COMBINATIONS TO INFECTION BY STRAINS OF THE TRISTEZA VIRUS[1]

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SUMMARY

Citrus plants grown under screenhouse conditions were inoculated with buds from 8 tristeza-infected citrus trees of different varieties introduced into Texas before 1943. Buds from 1 source tree caused severe tristeza symptoms on lime seedlings; buds from 7 sources caused mild symptoms. Some virus strains with mild effects on lime seedlings caused stunting of sour orange seedlings; others did not. The response of tristeza-infected Valencia orange and Red Blush grapefruit trees on sour orange and Cleopatra mandarin rootstock varied with rootstock and strain of tristeza virus.

Prior infection of lime seedlings by mild strains from 6 sources did not inhibit development of severe symptoms from infection with a severe strain, whereas a mild strain from 1 Meyer lemon tree did. When lime scions carrying the mild strain of virus from this Meyer lemon source were grafted on lime seedlings carrying the severe strain, the growth from the scion showed no recognizable severe symptoms during a 20-month period of observation. However, some lime seedlings inoculated by tissue from the tops of the grafted plants showed severe symptoms. When lime seedlings were inoculated simultaneously with mild and severe strains of tristeza virus, the position of the inoculum buds and the source of the mild strain affected the occurrence of severe symptoms.

The discovery of mild and severe strains of tristeza virus in citrus introductions into Texas (16, 17) provided an opportunity to study the response of citrus seedling and newly-planted commercial trees to bud-inoculated tristeza virus from different field sources. Studies were conducted under screenhouse conditions with grapefruit and orange trees, and sour orange and Mexican lime seedlings. This article also reports on the response of lime seedlings bud-inoculated with a severe strain when the seedlings were *a*) previously infected or *b*) simultaneously infected by a mild strain, and *c*) when scions carrying a mild strain providing cross-protection were grafted to rootstocks carrying the severe strain of tristeza.

VIRUS STRAINS AND MIXTURES REPORTED FROM DIFFERENT REGIONS.—The existence of tristeza virus strains has been demonstrated in Brazil (4, 7, 8), Africa (10, 12), and Texas (17), and in Florida (9), where different strains were separated from naturally infected plants. Fraser (6) and McClean (13) indicated that, in Australia and South Africa, the virus content of different trees, and particularly trees of different citrus species, may differ qualitatively. Grapefruit seedlings were selective in the virus that they acquired naturally, and they possessed some defensive mechanism against the more harmful component of the full virus complex (14). Sometimes aphids transferred only a part of the whole complex, and the part transferred induced either a milder reaction or no reaction at all, depending on the species of the test seedling (7, 13). In Florida, a milder form of tristeza virus was transmitted from Meyer lemon by aphids than by tissue grafts (15).

In Brazil initial evidence indicated that plants invaded by the mild strain of tristeza virus were protected against the severe strain (7). In later studies, growth made by 4 types of scions on sour orange rootstock was very poor when they were inoculated with the severe strain; growth of these scions on the same rootstock was less adversely affected by mild strains (4). In Brazil, plants simultaneously inoculated with mild and severe strains of tristeza virus produced growth intermediate between that caused by the mild or severe strains alone (3). In Texas, prior infection by a mild strain from a Meyer lemon tree inhibited the severe symptom development in lime plants inoculated with the severe strain of virus from Sueoka satsuma (17). In Florida the cross protection provided by prior inoculation with mild strains ranged from little to considerable; a lack of complete distribution of any single virus strain in lime plants offered a possible explanation for the variation in protective effects (9).

CLASSIFICATION OF STRAINS ON THE BASIS OF SYMPTOMS PRODUCED ON LIME SEEDLINGS.—The virus strains used in the experiments reported herein were taken from Mexican lime seedlings previously inoculated from the tree sources described in Table 1. It was not known whether other viruses were present concurrently with tristeza in the 8 source trees.

Severe strain.—Mexican lime seedlings inoculated from Sueoka satsuma showed vein clearing, yellowing, and defoliation of leaves on terminal twigs, extensive stem pitting, thickened light-colored gum-impregnated bark of cheesy consistency, stunted growth, and a weak root system. Leaves on flushes sometimes showed corky enations along the veins on the upper sides of the leaves 3–6 months after initial vein clearing. The foregoing symptoms are hereinafter referred to as severe symptoms of tristeza; the virus causing them is referred to as the severe strain.

Mild strain.—Mexican lime seedlings inoculated from most tristeza-infected sources listed in Table 1 made vigorous growth after initial vein clearing. The leaves remained large and green, terminal twigs did not defoliate, some stem pitting occurred under bark of apparently normal thickness, and a strong root sys-

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tem was present. No vein corking appeared on the leaves. The foregoing symptoms are hereinafter referred to as mild symptoms of tristeza; the virus causing them is referred to as the mild strain.

Since mild strains from different host varieties differed in the responses they caused in these tests, the varietal name of the original virus-host plant is used with mild strain.

EXPERIMENTS.—*Response of sour orange seedlings to bud-inoculated tristeza virus from different sources.*—Fraser (6) and McClean (13) used Seville sour orange and Eureka lemon seedlings as indicator plants for the presence of a "seedling yellows" reaction associated with a strain or component of tristeza virus. Wallace (19), using Eureka lemon seedlings as indicator plants, found that a Meyer lemon in California was carrying a virus that caused the "seedling yellows" reaction. In Texas the severe strain of tristeza virus severely stunted sour orange seedlings; the Matsui-mild strain did not (17). Since the Texas citrus industry is based on use of the sour orange rootstock, it seemed desirable to determine the response of sour orange seedlings to infection by mild strains of tristeza from other sources.

Sour orange seedlings, about 8 in. tall, growing in 1-gal. cans of soil, were bud-inoculated with the severe strain and with the 7 sources of mild strains listed in Table 1. These inoculated seedlings, except for 3 that died, were set out at a 2 × 5-ft. spacing. Although only a small population was tested, the results (Table 1) indicate that some virus strains that cause mild symptoms on lime seedlings stunt sour orange seedlings; others do not.

Response of budded grapefruit and orange trees to bud-inoculation with various strains of tristeza virus.—Old-line Valencia orange and Red Blush grapefruit trees on Cleopatra mandarin and sour orange rootstocks were planted in October, 1954, at 5 × 6-ft. spacing in the screenhouse. Eight trees of each of the 4 top-rootstock combinations, arranged in a modified Latin Square design, were planted. The objective of the experiment was to compare the response of tristeza-infected trees with healthy trees on 4 scion-rootstock variety combinations. In February, 1955, 4 of the 8 trees of each top-rootstock combination were inoculated with the Valencia mild strain. At the same time, single trees of each of the 4 scion-rootstock variety combinations were inoculated with the Meyer A mild strain and other single trees were inoculated with the severe strain. In September, 1955, twigs from the various inoculated trees were grafted to Mexican lime seedlings. Transmission to the lime seedlings demonstrated that the top-rootstock combination trees had been infected with tristeza virus; grafts from trees inoculated with the severe strain induced corresponding severe symptoms on the lime seedlings, and the remainder only mild symptoms.

The relative sizes of the grapefruit and orange trees 31 months after inoculation are shown in Table 2. There was no significant difference in size between check trees and trees inoculated with the Valencia mild strain. Trees on sour orange rootstock infected with the severe strain or with the Meyer A mild strain were stunted, but the treatments were not replicated. Trees on Cleopatra mandarin rootstock and infected with the severe strain or the Meyer A mild strain showed some stunting, but absence of replications limits the significance of this comparison.

Another planting was made to provide replicated comparisons of the effect of virus strain on the growth of bud-inoculated grapefruit trees. Uninoculated controls were not used, since the previous test had shown no difference in size between uninoculated trees and trees infected by the Valencia mild strain. Thirty-six old-line Red Blush grapefruit trees, 18 on sour orange and 18 on Cleopatra mandarin rootstocks, were planted in the screenhouse at 5 × 3-ft. spacing in April, 1955. In August of the same year, 6 trees on each rootstock were inoculated with, respectively, the Meyer A mild strain, the Valencia mild strain, and the severe strain. The growth response of the inoculated grapefruit varied with the rootstock and with the different virus

TABLE 1.—*Trees in Texas found infected with tristeza virus in 1954 and used as virus sources in the present investigation*

Variety	Source of top	Rootstock	Year planted	Symptoms original top	Inoculated seedlings	
					Lime symptoms	Sour orange height [c]
Calamondin	Florida	Mandarin	1929	Pitting	Mild	16
Beauty of Glenn Retreat mandarin	Australia	Mandarin	1942	None	Mild	10
Meyer lemon A	China	[a]	1924	None	Mild [b]	19
Meyer lemon B	Unknown	[a]	1943	Stunting	Mild	26
Mediterranean blood orange	India	Mandarin	1942	None	Mild	12
Valencia orange	Florida	Sour orange	1929	None	Mild	27
Matsui satsuma	Japan	Citrange	1938	None	Mild	24
Sueoka satsuma	Japan	*Poncirus trifoliata*	1932	None	Severe	[d]

[a] Grown on its own roots.

[b] Prior infection by this virus strain inhibited the development of severe symptoms on Mexican limes reinoculated with the virus from Sueoka satsuma (17).

[c] Average height in inches of 3 plants after 12 months. Uninoculated controls were 27 in. tall.

[d] Plants died within 4 months. In other tests (17), sour orange, lime, and grapefruit seedlings infected by virus from Sueoka satsuma were severely stunted, whereas those infected by virus from Matsui satsuma were not.

TABLE 2.—*How inoculation with 3 strains of tristeza virus affected trunk cross sections[a] of 4 scion-rootstock combinations during 31 months in a screenhouse*

Scion-rootstock combination	No. of trees	Tristeza strain	Months after inoculation 6	12	31[b]	% increase
Valencia orange on sour orange	4	None	535[a]	934[a]	1899[a]	255
	4	Valencia mild	594	1070	2072	249
	1	Meyer A mild	435	535	894	106
	1	Severe	561	644	644	15
Valencia orange on Cleopatra mandarin	4	None	567	1066	1824	222
	4	Valencia mild	649	1099	2196	238
	1	Meyer A mild	484	1015	2635	444[c]
	1	Severe	472	796	2062	337[c]
Red Blush grapefruit on sour orange	4	None	603	1035	1708	183
	4	Valencia mild	621	1105	1971	217
	1	Meyer A mild	575	860	1204	109
	1	Severe	509	575	616	21
Red Blush grapefruit on Cleopatra mandarin	4	None	680	1134	2076	205
	4	Valencia mild	692	1175	2033	194
	1	Meyer A mild	644	1089	1887	193
	1	Severe	484	796	1719	255

[a] Area of cross section of trunk, 4 in. above bud union, in mm^2. The cross-sectional area of the trunk is considered the best single index of tree size.

[b] Thirty-one months after inoculation there were no significant differences between the sizes of uninoculated trees and trees inoculated with a Valencia mild strain. Statistical comparisons of the effect of other virus strains on tree size are not valid, since the treatments were not replicated.

[c] Trees were in a favored position, next to a water hydrant.

strains (Table 3). Trees on sour orange rootstock inoculated with the severe strain were stunted; those on Cleopatra mandarin were not. Indexing the inoculated grapefruit back onto lime seedlings resulted in typical severe or mild symptoms, according to the respective sources.

Response of lime seedlings previously infected by a mild strain to reinoculation with a severe strain.—Prior infection of lime seedlings by the Meyer A mild strain inhibited the development of severe symptoms expected from subsequent infection by a severe strain, indicating that these 2 strains were related (17).

To determine whether other mild strains also were related to the severe strain, lime seedlings growing in 1-gal. cans of soil were inoculated with the single severe strain and with the 7 mild strains listed in Table 1. One month later, when infection had taken place, as indicated by vein-clearing on the new leaf growth, some plants of each series were reinoculated with the severe strain. The plants were later transplanted to the soil in the screenhouse at 1 × 6-ft. spacing, with 3 replications of 2-plant groups for each treatment.

The response of the lime seedlings to the original infection with a mild strain or subsequent infection with

TABLE 3.—*How inoculation with 3 strains of tristeza virus affected trunk cross sections[a] of Red Blush grapefruit trees during 24 months in a screenhouse*

Rootstock and statistical indices	No. of trees	Tristeza strain[b]	Months after inoculation 0	24	% increase
Sour orange	6	Valencia mild	206	791	284
	6	Meyer A mild	234	627	170
	6	Severe	221	394	78
F value			NS	*	
LSD 5%				259	
LSD 1%				369	
Cleopatra mandarin	6	Valencia mild	623	1241	99
	6	Meyer A mild	639	1456	128
	6	Severe	556	1116	100
F value			NS	*	
LSD 5%				210	
LSD 1%				299	

[a] Area of cross section of trunk, 4 in. above bud union, in mm^2, the average of 6 plants: 2-plant groups in 3 replications. The cross-sectional area of the trunk is considered the best single index of tree size.

[b] Uninoculated controls were not included, since a previous trial (Table 2) had shown no significant difference in size of uninoculated trees and those inoculated with the Valencia mild strain.

a severe strain is shown in Table 4. Of the 7 sources of mild strain virus, only the Meyer A mild strain inhibited the increased severity of symptom expression of the severe strain. Index tests from each of the doubly-inoculated trees resulted in symptoms comparable in severity with those in the source tree.

Mild strain sources from tristeza-infected Meyer lemon trees different from those listed in Table 1 were also used in cross-protection studies on lime seedlings. Some inhibited symptom expression of the severe strain on lime seedlings; others did not.

Leaf pieces were taken from some Meyer lemon shoots that grew from the buds used to inoculate lime seedlings with a mild strain; the sampling was done several months after the seedlings had been reinoculated with the challenging severe strain. The Meyer lemon leaf pieces, from 2 different sources and originally infected with a mild strain, caused different reactions in lime seedlings; leaf pieces from Meyer B caused severe symptoms on the lime seedlings, whereas that from Meyer A did not. These results indicated that the severe strain used in reinoculation had moved into the foliage of the shoot grown from the original inoculation bud of Meyer lemon B in the presence of its mild strain, but not into similar foliage of Meyer A in the presence of its mild strain.

On the basis of the data in Table 4, there are at least 3 distinct tristeza strains: *a*) a severe strain, *b*) a mild strain that inhibits symptom expression by the severe strain (the Meyer A mild strain), and *c*) a mild strain that does not (the Valencia mild strain). These strains also varied in their effect on grapefruit and sweet orange trees grown on sour orange rootstock (Tables 2 and 3).

Response of lime seedlings to simultaneous inoculation with mild and severe strains.—Costa (3) made simultaneous inoculations of orange trees on sour orange rootstock with mild and severe strains of tristeza virus. He found that the growth of the double-inoculated trees was intermediate between the growths of trees inoculated with either the mild or the severe strain; there was no exclusion of one or the other virus, according to position of the bud, as described by Kunkel (11), working with peach viruses. Ross (18) considered that, when a plant is inoculated simultaneously with 2 or more viruses to which it is susceptible, all the viruses will usually cause infections initially, regardless of how closely they are related.

TABLE 4.—*Response of Mexican lime seedlings to inoculation with mild strains of tristeza virus in June, 1955, and with a severe strain in July, 1955*

Strain pairs	Seedling response, Jan., 1957	
	Symptom	Height[a]
Calamondin—none	Mild	49.5
Calamondin—severe	Severe	29.8
Beauty of Glenn Retreat mandarin—none	Mild	39.0
Beauty of Glenn Retreat mandarin—severe	Severe	30.3
Matsui satsuma—none	Mild	52.2
Matsui satsuma—severe	Severe	27.3
Mediterranean blood orange—none	Mild	35.8
Mediterranean blood orange—severe	Severe	27.0
Meyer lemon A—none	Mild	41.0
Meyer lemon A—severe	Mild[b]	40.0[b]
Meyer lemon B—none	Mild	39.2
Meyer lemon B—severe	Severe	28.9
Valencia orange—none	Mild	54.3
Valencia orange—severe	Severe	27.3
CONTROLS:		
Severe—none	Severe	19.0
None—severe	Severe	21.2
None—none	None	60.2

[a]Seedling height, in in., is the average of 6 plants: 2-plant groups in 3 replications. The LSD 1% for effect of virus strain and source on plant height: single-inoculated plants, 11.9 in.; double-inoculated plants, 9.4 in.

[b]This is the only case where symptom expression from a severe strain appeared inhibited by previous inoculations by a mild strain.

To determine the effect of location on the inoculum bud, lime seedlings in 1-gal. cans of soil were bud-inoculated simultaneously with mild and severe strains. Shoots growing from the inoculum buds, placed about 2 in. apart on the stems, were not pruned off. Shoots from severe-strain-infected buds were fewer in number and slower in development. Severe symptoms developed more rapidly and in more lime seedlings when the upper-bud inoculum carried the severe strain than when the lower-bud inoculum carried it (Table 5).

TABLE 5.—*Percentage of lime seedlings developing severe symptoms from simultaneous inoculation with mild and severe strains of tristeza virus*

Inoculum in upper position	Inoculum in lower position	No. of plants	Months after inoculation			Height at 13 months (in.)
			6	12	19	
Severe	Mild (Meyer A)	9	44	44	56	21.8
Mild (Meyer A)	Severe	10	10	20	20	27.7
Severe	Mild (Matsui)	8	100	100	100	20.8
Mild (Matsui)	Severe	8	50	50	75	21.6

[a]All inoculated plants showed tristeza vein-clearing symptoms after 6–10 weeks.

TABLE 6.—*Response of lime seedlings to subinoculation with leaf pieces from the shoots of mild-strain-infected buds of lime trees that 6 months previously had been inoculated simultaneously with mild and severe strains of tristeza virus*

Inoculum in upper position	Inoculum in lower position	1 year after subinoculation		
		No. of trees	Number with severe symptoms	Height (in.)
Severe	Mild (Meyer A)	12	9	8
Mild (Meyer A)	Severe	8	0	27
Severe	Mild (Matsui)	9	8	8
Mild (Matsui)	Severe	5	5	8

When the upper-bud inoculum carried the mild strain, severe symptoms were more prevalent with the Matsui mild strain than the Meyer A mild strain.

Later, leaves were collected from lime shoots growing from mild-strain-inoculum buds on the simultaneous seedlings. These were grafted to lime seedlings for indexing. The severe strain had moved from the upper-inoculum bud containing the severe virus strain to the foliage developing from the lower inoculum bud, which originally carried only the Meyer A or Matsui mild strain. The severe strain also moved in the reverse direction, into the shoot from the Matsui mild strain but not into that of the Meyer A mild strain, when inoculum buds were transposed (Table 6). Thus, both the point of inoculation and virus strain relationships affected the prevalence of the strains in lime seedlings inoculated simultaneously.

Response of lime scions carrying Meyer A mild strain when grafted on a rootstock carrying the severe strain.—Lime seedlings growing in 1-gal. cans and infected with the severe tristeza virus strain were side-grafted with 2- to 4-in. lime scions that carried Meyer A mild strain. Scions carrying the Meyer A mild strain were used because prior infection by this strain inhibited symptom expression by the severe strain (Table 2). The trees were later pruned so that the scions formed the top.

The 13 grafted plants were observed for 20 months, but no symptoms of the severe tristeza strain were noted in the growth from the scions. Four months after grafting, leaf-pieces or buds of growth from the scions were indexed on lime seedlings. Of 37 lime seedlings inoculated in this manner from 8 different plants, 25 showed definite severe symptoms and 12 showed only symptoms of the Meyer A mild strain. These results indicate that the severe strain moved from the lime rootstock to the Meyer-A-mild-strain-infected growth resulting from the scion, even though the top showed only mild symptoms during the 20-month observation period. Thus, absence of recognizable severe symptoms in mild-strain-infected tops on severe-strain-infected rootstock did not mean absence of the severe strain in foliage and twigs of the top.

An attempt to grow similar plants with a severe-strain-infected top and a Meyer-A-mild-strain-infected rootstock was unsuccessful because shoot growth did not develop from the scions; sucker growth was abundant from the rootstock.

These results are in agreement with studies of some other virus strains infecting other crop plants. Where prior infection with one strain protects against further invasion by a related strain, the presence of one strain does not seem to prevent the entry of another, but does prevent multiplication sufficient to produce symptom dominance (1).

DISCUSSION.—Although tristeza virus has been widely distrubuted in infected Meyer lemon trees, there has been no apparent natural spread of tristeza in commercial plantings of grapefruit and sweet orange on sour orange rootstock in Texas. Present indications are that insect transfer of tristeza virus does not occur or is not common in the field (5). Thus, the absence of injury from tristeza in commercial Texas citrus is ascribed to absence of an efficient vector, not to the mildness of the virus strains present in Meyer lemon and other citrus importations. The apparent absence of an efficient insect vector in Texas also made it impossible to use an insect vector to separate tristeza virus from other possible contaminant viruses. Thus, some of the virus-source trees listed in Table 1 may carry viruses other than tristeza.

These trials supplement existing evidence that strain differences may be expressed as differences in severity of symptoms on infected lime and sour orange plants, and as differences in degree of stunting of citrus varieties on different rootstocks, and as different responses to cross-protection tests. If tristeza is defined as a decline desease of sweet orange trees on sour orange rootstock, then the Valencia mild strain—which caused no visible injury to that combination under screenhouse conditions—cannot be classified as a strain of tristeza virus on the basis of these tests. If tristeza virus is considered to be one that causes different degrees of vein-clearing and stem-pitting symptoms on lime seedlings, then the evidence presented here indicates the existence of several strains of the tristeza virus, some of which can infect sweet orange trees on sour orange rootstock without producing visible symptoms in screenhouse tests. The fact that infection with some mild strains does not protect against symptom expression by later infection with a severe strain may have 2 explanations: *a*) the severe strain may consist of 2 or more viruses, one of which is different from the mild strain; or *b*) the mild strain, even though related to the severe strains, for some reason behaves like curly top virus (2) and does not produce cross-protection against the severe strains. The results presented here are compatible with either concept, but the latter seems preferable.

CITRUS ROOTSTOCK INVESTIGATIONS
U. S. DEPARTMENT OF AGRICULTURE
AND
TEXAS AGRICULTURAL EXPERIMENT STATION
WESLACO, TEXAS

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LISTING OF BOOKS

Lucas, George B. 1958. **Diseases of Tobacco.** The Scarecrow Press Inc. New York, 498 p. Price $10.00.

This book will prove useful to most students and research workers in plant pathology because of the thorough and painstaking job that the author has done in assembling basic information on the etiology and control of the diseases of tobacco, many of which are important in other crops. The book is divided into 8 parts: 1) General (discussion of the world-wide tobacco-growing industry with examples of losses caused by diseases and chapters on disease control in relation to plant bed management, crop rotation and disease resistance); 2) Nematode diseases; 3) Fungus diseases; 4) Bacterial diseases; 5) Virus diseases; 6) Diseases caused by flowering plants; 7) Malnutritional diseases; and 8) Miscellaneous diseases. The pertinent literature on each important disease has been reviewed and discussed thoroughly and a list of references is given after each chapter. The literature lists are comprehensive, complete, and up-to-date, including references to literature published as recently as 1957.

Symposium on latency and masking in viral and rickettsial infections. 1958. Edited by Duard L. Walker, Robert P. Hanson, and Alfred S. Evans. Burgess Publishing Co., Minneapolis, Minn. 202 pp. Price $4.75.

This volume reports the proceedings of a conference held at the University of Wisconsin Medical School, September 4, 5, and 6, 1957. It contains 16 papers under general headings as follows: 1) Viral Characteristics Leading to Masking and Latency, 2) Factors of Host Cell and Environment Contributing to Masking and Latency, 3) The Role of Inhibitors in Masking and Latency, and 4) Consideration of Concepts and Terminology of Viral Infection. Reported also are the condensed discussions which followed presentation of papers on these subjects. Of particular interest to plant pathologists are the papers on "*Possible Host-Virus and Cell-Virus Relationships*," by C. H. Andrewes; "*Interrelation between Bacteria and Bacteriophage*," by André Lwoff; "*Bearing of Virus Mass on 'Masking' and Latency*," by J. W. Beard; "*Gradations and Transitions between Pathogenicity and Commonsalism in Infection with Plant Viruses*," by F. C. Bawden.

JOHN WILLIAM ROBERTS, 1882-1957

John C. Dunegan

JOHN W. ROBERTS, a charter and life member of the Society, and his wife were killed in an automobile accident in Florida on November 15, 1957. With these fateful words, we write finis to the life and career of a scientist who had a profound influence on the development of fruit pathology in the United States from 1911 to 1945.

Born in Alma, Nebraska, and a graduate of the University of Nebraska, John W. Roberts joined the staff of the Department of Agriculture in 1911. He was soon involved in studies on the apple bitter rot fungus and the frog-eye leaf spot of apples, both moot problems at the time. He described the manner in which the bitter rot fungus overwinters and in a series of simple but carefully planned experiments proved that the frog-eye symptom was caused by *Alternaria mali*, a secondary fungus that invaded areas previously killed by the black rot fungus. Later he initiated studies on the control of the bacterial spot disease of peaches. He was the first to show that proper nutrition of the trees was an important facet in the control problem. The zinc-lime spray was developed as a part of this bacterial spot control project. He also studied the peach brown-rot fungus and its control over a period of many years, and initiated a project on the development of new fungicides. This work was started before the commercial companies entered this field and many of the pioneer field studies with the dithiocarbamates were carried out under his direction. Research on fungus and bacterial diseases of fruit trees was but a portion of his contribution to plant pathology. For many years he was a member of the Editorial Board of the Journal of Agricultural Research, a task for which he was preeminently qualified through his wide reading and unexcelled command of the English language.

After 36 years of service to plant pathology, he retired as principal pathologist in 1945 and moved with his much beloved wife to Florida. Their frequent letters revealed how much they mutually enjoyed the well deserved rest and relaxation of retirement. A precise thinker, friendly and endowed with a kindly sense of humor, John W. Roberts was ever willing to help his colleagues and younger workers with their problems.

It is a sad commentary on the pace of modern living that the end should come to this devoted couple through a highway accident. Yet, to those who knew them intimately and knew how inseparable they were, it seems fitting that they should die together—it is as they would have wished.

REPORT AND ABSTRACTS OF THE 1958 MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fifteenth annual meeting of the Potomac Division of the American Phytopathological Society was held February 27 and 28, 1958, in the auditorium of the Plant Industry Station, Beltsville, Maryland. Twenty-five scientific papers were presented. About 150 people attended this meeting. Following the banquet, on the evening of February 27, Dr. John L. Creech showed pictures he had taken while exploring for ornamental plants in Japan.

Officers elected to serve during the next year were: *President*, L. O. WEAVER; *Vice-President*, W. D. MCCLELLAN; *Secretary-Treasurer*, JOHN G. MOSEMAN; and *Councilor*, JOHN W. HEUBERGER.

JOHN G. MOSEMAN, *Secretary-Treasurer*

Host-parasite relationships of the pin nematode, Paratylenchus projectus, on tobacco and tall fescue. COURSEN, B. W., AND W. R. JENKINS. Effects of 1, 5, and 10 thousand specimens of *Paratylenchus projectus* used as inocula on Catterton variety of tobacco and Kentucky 31 variety of tall fescue were investigated. Dry weight of roots, height of above-ground portions, and size of nematode populations were determined at monthly intervals for 3 months. Inoculations of tobacco plants caused stunting of top growth, shortening of internodes, and a marked increase in size of root systems. Lateral roots, characteristically large on healthy plants, were not well developed on inoculated plants. Effects of *P. projectus* were similar on tall fescue. Tillering was 31% greater on plants inoculated with 10,000 nematodes than on uninoculated controls. Smaller nematode populations increased at a greater rate than larger ones in association with tobacco or fescue. Ninety-seven plant species and varieties representing 10 families supported populations of *P. projectus* for 60 days. Nematodes were not recovered from Chantenay variety of carrot, California Wonder variety of pepper, and Marketer and Palmetto varieties of cucumber.

Fungicides and the amino acid spectrum of fungus mycelium. CROSSAN, D. F., I. F. BROWN, JR., AND M. R. SIEGEL. *Botryosphaeria ribis* G. et D. and *Colletotricum capsici* (Syd.) B. et B. were grown in liquid culture with and without sub-lethal amounts of captan (4 ppm) and copper (6 ppm) fungicides, respectively. Weighed portions of dried mycelia were hydrolyzed with 6*N* H_2SO_4, neutralized, and the hydrolysates reduced in volume for spotting on chromatographic paper. Hydrolysates were developed in 1 dimension in a butanol-acetic acid-water mixture (4:1:5) and in a second dimension in a mixture of meta-cresol and phenol (1:1). The paper was buffered with borate buffer (pH 9.3) for development in the second dimension. In the absence of captan, *B. ribis* contained cystine, lysine, aspartic acid, glycine, threonine, glutamic acid, proline, γ-amino butyric acid, tyrosine, methionine, valine, isoleucine, and leucine; in the presence of 4 ppm captan, threonine, glutamic acid, and γ-amino butyric acid were missing. In the absence of copper sulfate, *C. capsici* contained cystine, lysine, asparagine, aspartic acid, glycine, serine, threonine, alanine, glutamic acid, proline, γ-amino butyric acid, valine, methionine, and leucine; in the presence of 6 ppm copper sulfate, γ-amino butyric acid and methionine were missing and threonine and glutamic acid were reduced in quantity.

Histology and cytology of susceptible and resistant soybeans infected with Meloidogyne incognita acrita. CRITTENDEN, H. W. Three varieties of root-knot-nematode-susceptible soybeans (Virginia, Adams, and Hakote) and 5 resistant varieties (Blackhawk, Mukden, Anderson, Laredo, and Jackson) were exposed to *Meloidogyne incognita acrita.* Collections were made of roots at 4, 8, 16, and 32 days of age. Each of the 3 susceptible varieties exhibited the following characteristics: 1) giant cells surrounding the head of the nematode; 2) large number of giant cells; 3) large size of giant cell area; 4) very dense cytoplasm within giant cells; 5) great number of enlarged nuclei in each giant cell; and 6) great enlargement of pericycle, with giant cells occurring frequently in this region. The 5 resistant varieties exhibited a gradation of abnormal tissues and cells that corresponded to their degree of resistance based on field and greenhouse tests. Very resistant varieties (Laredo, Anderson) showed: 1) head of nematode not surrounded by giant cells; 2) small number of giant cells; 3) small size of giant cell area; 4) cytoplasm of giant cells sparse and not dense; 5) small number of enlarged nuclei in each giant cell; and 6) enlargement of pericycle small or none, with no giant cells in this region.

Weight loss of wood exposed to Merulius lacrymans Fr. under four environmental conditions. DILLER, J. D., AND E. J. KOCH. The influence of environment on the development of *Merulius lacrymans* Fr. was examined in a 1955 study at Beltsville, Maryland. This was part of a factorial experiment that also included variations in soil and fertilizer factors, and in pH reaction. Individual test blocks of 13 wood species, segregated into categories of heart-, sap-, and heart-sap-wood, were placed in 8-oz. cans, inoculated with the fungus, and grown in the following 4 environments: 1) in the crawl-space area of a 2-family, occupied, basementless dwelling; 2) under a mound of leaves in a wooded area; 3) in an abandoned root cellar; and 4) in a 40°F temperature-controlled room. Small amounts of water were added to the samples when needed. After 24 months the weight loss of each block was determined. Results show that environment plays a very important role in relation to weight loss caused by *M. lacrymans.* The average weight loss for the 468 blocks of wood stored in the 40°F constant-temperature environment was only 3.7% in comparison to a weight loss of 11.1% for the 468 blocks stored in the crawl-space of a basementless house. Weight loss averaged 7.4% for the root cellar and 8.9% for those stored under mounds of leaves.

Host-parasite relationships of Avena sativa and a root-knot nematode, Meloidogyne incognita acrita. FERVER, A. F., AND H. W. CRITTENDEN. Root-knot-nematode-resistant oat varieties (Arlington and Atlantic) and susceptible oat varieties (Lee and Forkedeer) were grown in the greenhouse in the presence and absence of *Meloidogyne incognita acrita.* Using one-dimensional paper partition chromatography, hydrolysates of non-infected roots of the 4 varieties were analyzed for amino acids and sugars. No qualitative differences in amino acids or sugars were noted between the 4 varieties. Infected roots stained with osmic acid revealed an enlargement of the stele and cortex in all varieties. Few galls or mature females were found in the resistant varieties. No nematodes were found completely embedded in the stele; only the head extended into the vascular tissues. Microsections of infected roots of the susceptible and resistant varieties showed giant cells and tissue distortion surrounding the nematode head. Aggregation of nuclei in the dense cytoplasm of the giant cell was observed. Microsections of 40-day-old non-infected roots of the varieties indicate that the inner tangential endodermis cell wall is thicker in the resistant varieties than in the susceptible varieties.

Indexing sour cherry trees for necrotic ring spot on excised twigs. FINK, HARRY C. Sour cherry trees can be indexed for necrotic ring spot virus by inoculating dormant twigs excised from them. A bud from a tree known to be infected with the virus is grafted between 2 axillary buds on the twig. The proximal end of the twig is immersed in a 3-ppm gibberellin solution. The twigs are held under conditions suitable for growth; tap water is added to the solution as required. Symptoms will appear on the newly unfolding leaves of twigs cut from ring-spot-free trees; conversely, symptoms will not appear on leaves of twigs cut from infected trees. An alternate method is to insert a bud from trees to be indexed on twigs cut from ring-spot-free trees. Symptoms indicate the tree being indexed as "virus" infected. The number of bud sticks to be used for each tree to be indexed depends upon the size of the tree, since the virus may not be distributed equally throughout a newly infected tree. One stick from a nursery tree should be sufficient. This method of indexing may prove valuable in disease surveys, virus spread studies, and nursery certification programs where large numbers of trees have to be indexed annually.

Differential response of alfalfa clones to variant forms of Corynebacterium insidiosum. FULKERSON, J. F. Seedling populations of alfalfa varieties are known to vary in susceptibility to bacterial wilt. Uniformly susceptible plants of vigorous growth habit were needed to determine the relative virulence of different isolates of the wilt organism *Corynebacterium insidiosum.* For this purpose 150 seedling plants of DuPuits, a wilt-susceptible alfalfa variety, were each vegetatively propagated. These clones were then tested for susceptibility to a wild-type isolate of the causal organism, using root-inoculation techniques. After 2 such tests, 4 clones (9, 75, 83, and 146) were selected, each having a known degree of susceptibility to the wild-type isolate. In tests using these clones, severe crown symptoms developed within 44 days of inoculation of the plants with the wild-type isolate. In comparative tests with a culture variant of the wild-type isolate, clone 83 was significantly more susceptible to the variant form than to the wild-type isolate. Clones 9, 75, and 146 were equally susceptible to both isolates. The variant form was indistinguishable in culture from some isolates obtained from naturally infected field-grown alfalfa plants.

Fungicides and the control of Botryosphaeria ribis fruit rot of apple. HEUBERGER, J. W. In 1957 a block of the Rome variety was used for a large-scale field test. The spray schedule was divided into 2 parts: A—pre-pink through first cover; B—fourth cover to harvest. All plots

except Cyprex and glyodin received captan (1½ lb.) at the second and third cover. Fungicides were: 1) Cyprex (1 lb.), all season; 2) glyodin + organic mercury (1½ pt.–¾ pt.) in A, and Thioneb (1½ lb.) in B; 3) Phybam-S (4 lb.); 4) Magnetic 70 sulfur (6 lb.); 5) Magnetic 70 sulfur + captan (3 lb.–1½ lb.); and 6) captan (2 lb.) in A, each followed by 2 lb. captan + zineb (1:1) in B. Applications were made by a speed sprayer. *Botryosphaeria ribis* became epiphytotic. Counts of 200 fruit on each of 4 trees per plot and on all dropped fruit were made at bi-weekly intervals, beginning August 1 and ending October 11. Best control was shown by captan, Magnetic 70 sulfur + captan, and Phybam-S in A, followed by captan + zineb in B (0.5% infection on tree and 12% on drops, compared to 17% and 70%, respectively, for unsprayed). At harvest, fruit from each plot was brushed, graded, boxed, and placed in cold storage (32°F) for 94 days and then removed to room temperature (70°F±) for 7 days. Percentage rot in the unsprayed was 54, compared to 9, 18, and 15 for the 3 best schedules.

Time of apple fruit infection by Gloeodes pomigena and Microthyriella rubi. Hickey, K. D., F. H. Lewis, and C. F. Taylor. The time of apple fruit infection by the sooty blotch (*Gloeodes pomigena* (Schw.) Colby) and fly speck (*Microthyriella rubi* Petr.) fungi was studied under epiphytotic conditions. Rome Beauty apples were bagged in West Virginia on June 12, 1950. The bags were removed at intervals up to harvest. Sooty blotch infections took place from June 22 until harvest, and either inoculation or infection may have occurred before June 12. Fly speck infections took place from June 22 through August. A second test was conducted, in which the fruit was bagged on various dates and kept covered until harvest. The results were comparable to those from the first test. In Pennsylvania, in 1956, 1400 Grimes Golden and Stayman Winesap apples were bagged on June 21. Lots of 200 apples were exposed for each succeeding half-month interval, and then bagged again. Very heavy infection occurred during any favorable weather period from late June through mid-September. Infection may have occurred before June 21. These data show that sooty blotch and fly speck infections may occur within about 1 month after petal-fall, and continue at a high rate into September. Late infections may be unnoticed because of inadequate time for symptom expression.

Natural transmission of sweet potato feathery mottle virus. Hildebrand, E. M. By use of Porto Rico variety of sweet potato as indicator, feathery mottle virus was found to spread naturally during 1955–1957 at Beltsville, Md. Apparently, conditions did not favor the vector in 1955 and 1956, because the percentages of natural transmission were but 8 and 6, respectively, compared with 23.5 in 1957. A climatic aberration late in the season, however, might account for the increase in 1957. The severe drouth up to late August resulted in poor growth. A heavy rain, followed by unusually warm weather, in September produced vigorous growth, and some of the indicator plants exhibited feathery mottle symptoms on the terminals, something hitherto unobserved. In the test planting, consisting of 7 100-foot rows, the feathery mottle inoculum was furnished by single rows of Porto Rico and a Russian sweet potato (P.I. 105945), both of which were infected also with internal cork virus. The greenhouse "flush of growth" revealed a mean average of 28 per cent natural transmission for the 5 rows, with values of 21 and 26 for Porto Rico, and 23, 32, and 36 for seedling clone XXI (open-pollinated Porto Rico). Of high susceptibility to feathery mottle and high resistance to internal cork, clone XXI is proving an invaluable aid in separating these syndromes.

Morning glories as indexing hosts for sweet potato viruses. Hildebrand, E. M. From 1955 to 1957, morning glory varieties were tested for susceptibility to sweet potato viruses. Many produced symptoms of internal cork virus; very few produced symptoms of feathery mottle virus. Being transmissible only by grafting, the latter presents much difficulty, for morning glory is poorly adapted for this purpose and the natural vector is unknown. The internal cork virus is readily transmissible by aphids, grafting, and mechanical means. The first test demonstrated 5 morning glory varieties (including Scarlett O'Hara) to be good indexing hosts for the internal cork virus (or viruses). The second and third tests included the world-wide collection of *Ipomoea* spp. of the New Crops Branch, Agricultural Research Service. From mid-September to mid-March the photoperiod had to be lengthened for suitable vegetative growth. With the rapid-transmission techniques (stimulating a new spurt of growth by pruning) the incubation periods for internal cork were usually 5–15 days. Symptoms included vein-banding, vein-clearing, chlorosis, chlorotic spot, dwarfing, stunting, dieback, and death. *Ipomoea* spp. were generally susceptible, whereas *Quamoclit* spp. and *Convolvulus* spp. were not. In general, morning glories were unsuitable for indexing feathery mottle.

Effective life of fungicides as a factor in the control of sooty blotch and fly speck on apple. Lewis, F. H., and K. D. Hickey. Control of sooty blotch, caused by *Gloeodes pomigena* (Schw.) Colby, and of fly speck, caused by *Microthyriella rubi* Petr., was obtained under epiphytotic conditions with ferbam, zineb, glyodin, and lead arsenate sprays ending in August, on apples harvested in October. Captan and thiram failed under these conditions. In fungicide-agar tests, greatest growth suppression was provided by ferbam, followed by captan and zineb. Glyodin and lead arsenate had little effect. In spore germination trials, glyodin, captan, zineb, and ferbam were more effective than lead arsenate. In an effort to explain the performance of captan and zineb, orchard sprays were discontinued at half-month intervals from petal-fall (May 14) to September 3. Captan controlled sooty blotch and fly speck until the last spray treatment, after which visible sooty blotch lesions appeared in 35–40 days. Zineb gave control for 60 days. Residue data did not offer an adequate explanation for the rapid failure of captan. The effective life of the spray residue seems to be the major factor involved in these results. The performance of lead arsenate, and perhaps thiram, can be explained on the same basis.

Effect of holding temperatures on brown and Rhizopus rots of hydrocooled peaches. McClure, T. T., and W. L. Smith, Jr. Peaches inoculated with *Rhizopus stolonifer* (the Rhizopus rot fungus) or *Monilinia fructicola* (the brown rot fungus) were hydrocooled in water or in a 0.1 per cent Dowicide A solution, held 3 days at 40°, 45°, 50°, 55° or 60°F, and then placed at 75° for 3 more days. Dowicide A significantly reduced the amounts of both rots. The effectiveness of Dowicide A when the holding temperature was 60° was about half that when the holding temperature was 40°. The amounts of brown and Rhizopus rots that developed in all lots of peaches varied with the holding temperature and increased significantly in lots treated with Dowicide A when holding temperatures were greater than 50°. Holding temperatures above 40° significantly increased brown rot, and those above 45° significantly increased Rhizopus rot in lots hydrocooled in water.

Reactions of Fragaria vesca seedlings to a single strawberry virus isolate. McGrew, J. R. All stocks of the East Malling clone (EMC) of *Fragaria vesca* used as strawberry virus indicators apparently carry latent A virus. Since there is no evidence of seed transmission of strawberry viruses, 35 seedlings of EMC were grown. These were individually stolon-grafted to a virus isolate from the Suwanee variety of the type previously described as latent C. This isolate produces in the EMC type-2 symptoms of Demaree and Marcus. Nineteen seedlings developed distinct leaf and petiole epinasty, followed by dwarfing characterized as: very severe (in 4); severe, equal to this isolate in EMC (in 6); intermediate (in 8); and mild (in 1), even though latent A was absent. The 16 symptomless

seedlings were then grafted to EMC plants to determine whether they had been inoculated with C virus. Development of type-2 symptoms in the EMC plants of every graft indicated successful inoculation. Although latent A from the EMC plants could have moved reciprocally across the graft unions into these symptomless seedlings, none showed a reaction to C virus plus latent A. Expression of C virus apparently depends on the susceptibility of the particular *F. vesca* seedling clone rather than on the presence of a conditioning virus such as latent A.

Separation of genes conditioning resistance to Erysiphe graminis f. sp. hordei by two pathogenically different cultures. MOSEMAN, J. G., AND C. W. SCHALLER. The barley varieties Algerian (C. I. 1179), Ricardo (C. I. 6306), and Manchuria (C. I. 2330) were intercrossed and the progeny tested with the monoconidial cultures CR3 (California race 3) and 061 of *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal. Algerian was resistant to both cultures, Ricardo resistant only to culture CR3, and Manchuria susceptible to both cultures. Tests of the progeny with culture CR3 indicated that each of the resistant varieties possessed a single gene for resistance to culture CR3, and that the gene in Ricardo was either identical to, allelic, or less than 1 crossover unit from the Ml_a gene previously identified in the variety Algerian. Results with culture 061 indicated that the resistance in Algerian to culture 061 was also conditioned by a single gene. Identical reactions of F_2 progenies from the cross Algerian × Manchuria to both cultures demonstrated that the same gene, Ml_a, conditioned resistance to both races. Since Ricardo was susceptible to culture 061, the gene in Ricardo is not the Ml_a gene, but either an allele or a second gene less than 1 crossover unit away. Such a low value justifies considering the genes as alleles. The gene in Ricardo has been designated Ml_{ra}.

A new strain of tobacco streak virus. PATINO, G. A new virus producing primary and systemic necrotic symptoms on beans was isolated from peas collected in southern Idaho. Of 30 bean varieties tested, all were susceptible. Among 46 plant species tested, 29 species from the families Leguminosae, Solanaceae, Amaranthaceae, Portulacaceae, and Compositae were susceptible to the virus. The virus was inactivated between 62° and 64°C, after storage for 27 hours *in vitro* at room temperature, and it withstood dilution of 2000×, but not of 4000×. It was still infectious after 65 days of storage in dry plant tissue at room temperature. No evidence of seed transmission was found. Similarity of symptoms produced on beans and other hosts, physical properties, and cross-protection tests suggest that this virus is related to the viruses of red node and tobacco streak. The new virus can be distinguished from them in that it does not produce necrotic symptoms on tobacco and is not infectious to crimson clover, *Crotalaria* sp., cucumber, guar, red clover, and white sweetclover, all of which are infected by the tobacco streak and red node viruses. It also has a higher thermal inactivation point and withstands a higher dilution than these 2 viruses.

Some effects of certain antibiotics and other organic chemicals on the growth of sclerotium rolfsii in the laboratory. PORTER, FRANK M., AND CARROLL E. COX. Two means were used to evaluate the toxicity of 75 antibiotics and other organic chemicals to *Sclerotium rolfsii* Sacc. 1) Sclerotia were immersed in 500 ppm of the chemical for 24 hours before plating on agar. The per cent inhibition due to the chemical was computed by comparing the diameter growth of chemical treatments to the diameter growth of controls. 2) Inoculum blocks were placed on agar containing the chemical. After 1 week the growth or absence of growth was observed. All concentrations were computed as active ingredients. Among the compounds tested, Omadine disulfide and sodium *N*-methyldithiocarbamate (Vapam) completely inhibited the germination of sclerotia. At 1 and 100 ppm, respectively, these compounds also prevented mycelial growth. The zinc salt of Omadine and alpha alpha trithiobis(*N*-dimethylthioformamide) did not appreciably inhibit the germination of sclerotia but completely prevented mycelial growth at 1 and 10 ppm, respectively. Dodecylguanadine (Cyprex) prevented the germination of sclerotia but did not appreciably retard the growth of mycelium at 2000 ppm. Pentachloronitrobenzene (PCNB) did not completely prevent the growth of mycelium although growth was markedly retarded. Thiolutin and Actidione completely prevented the growth of mycelium at 15 and 25 ppm, respectively.

Microclimate and tomato anthracnose. RANIERE, L. C., AND D. F. CROSSAN. This study was conducted to determine the influence of microclimate in irrigated and non-irrigated tomato plots on *Colletotrichum phomoides* (Sacc.) Chester. On 5 consecutive days following a given irrigation, a series of measurements was obtained on relative humidity, dew formation, and temperature within both irrigated and non-irrigated tomato plants through the use of a hand-aspirating psychrometer, 2 dew meters, and 2 hygrothermographs. Data obtained indicate that temperature within the plant foliosphere was not greatly affected by irrigation. Relative humidity readings revealed considerable differences for as long as 5 days after an irrigation, the relative humidity within the foliosphere of irrigated plants frequently exceeding that of non-irrigated plants by 20%. Irrigation also influenced the duration and intensity of dew formation, the dew period being extended for as many as 4–6 hours in irrigated plots over that in non-irrigated plots. A subsequent increase in the severity of tomato anthracnose was recorded in the irrigated tomato plots. Laboratory studies indicate that *Colletotrichum phomoides* spores will germinate in the absence of free moisture at 92% relative humidity, and that a 12-hour exposure to relative humidity below 80% resulted in a decrease in spore viability.

The chemical basis of resistance of asparagus to the nematode Trichodorus christiei. ROHDE, R. A., AND W. R. JENKINS. Asparagus (*Asparagus officinalis* L. Hort. var. Mary Washington) would not support populations of *Trichodorus christiei* for more than 40–50 days. Decline of populations on young plants was at the same rate as starvation in sterile soil, but after fleshy storage roots had formed, population decline was more rapid. Tomato, normally a good host, supported only very low nematode populations when asparagus was present in the same pot. *T. christiei* did not feed on asparagus after it had passed the young seedling stage. Juice from asparagus roots was toxic to individuals of *T. christiei* and several other nematode species when diluted 1:10, while tomato root juice was not toxic. A toxic compound was isolated from this juice by extraction with butanol and ethanol, followed by ion exchange and paper chromatography. The final product was a light-brown powder having an approximate molecular weight of 148. A proposed empirical formula is $C_6H_{12}O_6$. The product was carbohydrate-like and was obtained from root leachates as well as the roots themselves. The toxic compound caused non-reversible paralysis of nematodes in water solutions of 100 ppm or less. Populations of *T. christiei* around tomato roots were reduced by either spraying the leaves or drenching the root zone with 1000-ppm solutions of this compound.

Further studies on the host range of Aphanomyces cochlioides. SCHNEIDER, C. L. Seedlings of 94 plant species representing 31 families were exposed to zoospores of *Aphanomyces cochlioides* in Petri dishes and in pots of soil. The following species became infected: *Tetragonia expansa* (Aizoaceae); *Amaranthus blitoides, A. retroflexus, Celosia argentea, Gomphrena globosa* (Amaranthaceae); *Dianthus chinensis, Lychnis alba, Saponaria officinalis, S. ocymoides* (Caryophyllaceae); *Beta maritima, B. patellaris, B. trigyna, Chenopodium album, Kochia scoparia, Salsola Kali, Spinacia oleracea* (Chenopodiaceae); *Papaver Rhoeas* (Papaveraceae), and *Portulaca oleraceae* (Portulacaceae).

In addition, the fungus was isolated from plants of each of these species grown in naturally infested field soil. Older plants, as well as seedlings of the following common weeds and cultivated plants, were susceptible to infection: *Amaranthus blitoides, Chenopodium album, Salsola Kali, Saponaria officinalis,* and *Spinacia oleracea;* whereas the following were susceptible in the seedling stage only: *Lychnis alba* and *Portulaca oleracea.* Single-spore isolates from *Chenopodium album, Saponaria ocymoides, Spinacia oleracea,* and *Tetragonia expansa* caused damping-off and typical symptoms of black root on sugar beets.

Taxonomy, etiology, and control of several Anthracnose fungi attacking pepper. SMITH, R. W. *Capsicum annuum* L. is parasitized by several melanconiaceous fungi. *Colletotrichum* (*Gloeosporium*) *piperatum* (Ell. & Ev.) Ell. & Hals, or *C. nigrum* Ell. & Hals, causes what is commonly known as "anthracnose disease," whereas *C. capsici* (Syd.) B. & B. incites "ripe rot disease." *C. nigrum* is considered synonymous with *C. piperatum.* During certain years, anthracnose has caused appreciable losses in the market value of pepper fruit in Delaware. Fruit symptoms of anthracnose and ripe-rot are similar, but are distinquishable by the presence of black setae within the acervuli of *C. capsici.* Spore size and shape further distinguish the 2 fungi. After initially invading the flesh of the pepper fruit, both fungi may grow into the seed cavity and infect the seed. Survival as stromatic mycelium on dry harvested seed is possible for at least 9 months. Disease refuse can also serve as a means of overwintering for *C. piperatum.* Cross-inoculation experiments, using tomato and pepper, indicate that *C. piperatum* and *C. phomoides* (Sacc.) Chester are capable of attacking both hosts. In separate field plots, under severe disease conditions, zineb and maneb fungicides were found to give satisfactory control of *C. piperatum* and *C. capsici.*

Rhizopus decay of peaches as affected by post-harvest temperatures and moisture conditions. SMITH, W. L., JR., AND T. T. MCCLURE. Some peaches were wounded, inoculated immediately with *Rhizopus stolonifer,* and held 1–5 days at 32° or 40°F; other wounded peaches were held 1–5 days at 32° or 40° or 1 day at 75° before inoculation. Samples were kept at these temperatures in open containers or in perforated polyethylene bags with or without wet cotton over the wounds. They were then subsequently held uncovered at 75°, along with check fruit inoculated immediately after wounding, and held continuously at 75°. Peaches inoculated immediately and held at 32° or 40° before placement at 75° decayed less than those held continuously at 75°. Those held 3 or 5 days at the low temperatures decayed less than those held 1 day. Wounded peaches held uncovered 1–5 days before inoculation decayed less than similar ones in polyethylene, and less than those inoculated immediately after wounding and held at low temperatures before placement at 75°. Temperature between wounding and inoculation had little effect on decay. Peaches with cotton-covered wounds decayed almost as much as check fruit. Low temperatures presumably lessen decay by drying wounds rather than by affecting the virulence of Rhizopus.

A preliminary report on distribution of root-knot nematode species in the United States. TAYLOR, A. L., AND EDNA M. BUHRER. Since the separation of the species of the root-knot nematodes and their assignment to the genus *Meloidogyne* by Chitwood in 1949, information on the distribution of the species accumulated in the files of the Nematology Section of the U. S. Department of Agriculture indicates that: 1) The most common root-knot nematodes in the United States south of the latitude of Washington, D. C., are *Meloidogyne incognita* and its subspecies *M. incognita acrita.* 2) North of this latitude, *M. hapla* has been found out-of-doors more often than any other root-knot nematode species. 3) *M. arenaria* and *M. hapla* are fairly common in fields in the states where peanuts are grown, notably in Alabama, Georgia, and Virginia. 4) *M. javanica* has been collected from scattered locations in the southern and southwestern states. 5) *M. arenaria thamesi* has been found only in samples from Florida. 6) Other *Meloidogyne* species have not been found. 7) Interceptions by federal and state plant quarantine authorities indicate that root-knot nematodes have been repeatedly introduced into the United States from foreign countries with various kinds of planting materials, and that there has been considerable movement of root-knot nematodes from state to state. Thus, the finding of any *Meloidogyne* species in any part of this country can be easily explained.

A new virus-induced streak of pea. ZAUMEYER, W. J., AND G. PATINO. A new streak virus was isolated from several varieties of pea (*Pisum sativum*) in southern Idaho, where streak was widespread in 1956 and 1957. It produced on peas symptoms similar to those of the 3 previously described pea streak viruses, from Virginia, Wisconsin, and New Zealand. In the field the most conspicuous symptoms are necrotic streaking of the stems and necrotic spotting of the pods. Seriously infected pods are shrivelled and developed no seeds. Infected plants are slightly chlorotic, and the veins of the leaves and stipules are often cleared. Under greenhouse conditions a reddish-brown necrosis appears on the stems and petioles. Necrosis is usually most conspicuous at the nodes. Small necrotic spots and slight veinal necrosis are often noted on certain varieties. Many varieties show vein clearing of leaves and stipules. Leaf mottle has not been observed. The 115 varieties inoculated with the virus were all susceptible. The new streak virus differs in host range and physical properties from the other 3 described streak viruses of pea. It is inactivated at 65–70°C, at 1:10,000 dilution but not at 1:5000, and after storage for 24–48 hours at 20°.